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MOSAIC DISEASES IN THE CANARY ISLANDS, WEST AFRICA, AND GIBRALTAR[1]

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INTRODUCTION

The Allison V. Armour expedition was conducted primarily for the purpose of collecting living plants and seeds and introducing them into the United States. However, as the yacht used on the expedition was fitted for doing laboratory work and for growing potted plants, it was possible to conduct certain types of experiments coincident with the work of collection; and other experiments have been conducted since the return of the writer to the United States. On the expedition the writer's time was devoted to the search for plant viruses suitable for experimental studies and to the accumulation of information concerning the virus diseases of plants in the regions visited.

This report deals with the observations made in the Canary Islands, west Africa, and Gibraltar, and with some of the experimental results relating both directly and indirectly to the mosaic viruses from *Nicotiana glauca* R. Grah. collected on the Canary Islands and at Gibraltar. Limited comparative studies have been made with some of the viruses collected in the United States.

MOSAICS IN CANARY ISLANDS

The expedition reached Santa Cruz, Teneriffe, the capital of the Canary group, on December 22, and on December 28 it went to the island of Grand Canary. Several days were spent making observations and collections on each of these islands. During December the islands are rather cool and are rich in growing plants, including many kinds of garden vegetables.

Tomatoes and potatoes were growing in many localities, and various species of cucurbits were found in gardens and along the roadsides. Many of the potato plantings were located on narrow terraces, sometimes high above the highways and very inaccessible. In one of these terrace plantings which overlooked the sea near the port of Orotava, Teneriffe, green mosaic was found on most of the potatoes and on a garliclike plant[3] (fig. 1) which was growing as a

[1] Received for publication Mar. 2, 1929; issued October, 1929. The work described in this paper was done by the Allison V. Armour expedition of 1923-27. A full account of the two expeditions sponsored by Armour has been published by Fairchild (*1*).[4]

[2] The writer wishes to express his appreciation to Mr. Armour and to Doctor Fairchild for the opportunity of collecting and studying the mosaic diseases reported in this paper and for personal assistance rendered in various ways during the voyage. Credit is due to Dr. J. M. Dalziel, of Kew Botanical Gardens, Kew, England, who accompanied the expedition, and also to Oliver M. Freeman, of the United States Bureau of Plant Industry, for identifying some of the plants collected.

[3] When this plant was collected it was thought to be a wild garlic, and the writer referred to it as such in an abstract (*6*). After being studied further in a quarantine greenhouse, this plant appears to be one of the false garlics (*Nothoscordum fragrans* Kunth).

[4] Reference is made by number (italic) to "Literature cited," p. 577.

FIGURE 1.—Green mosaic on false garlic (*Nathoscordum fragrans* Kunth). Collected on the island of Teneriffe, Canary Islands. × 1

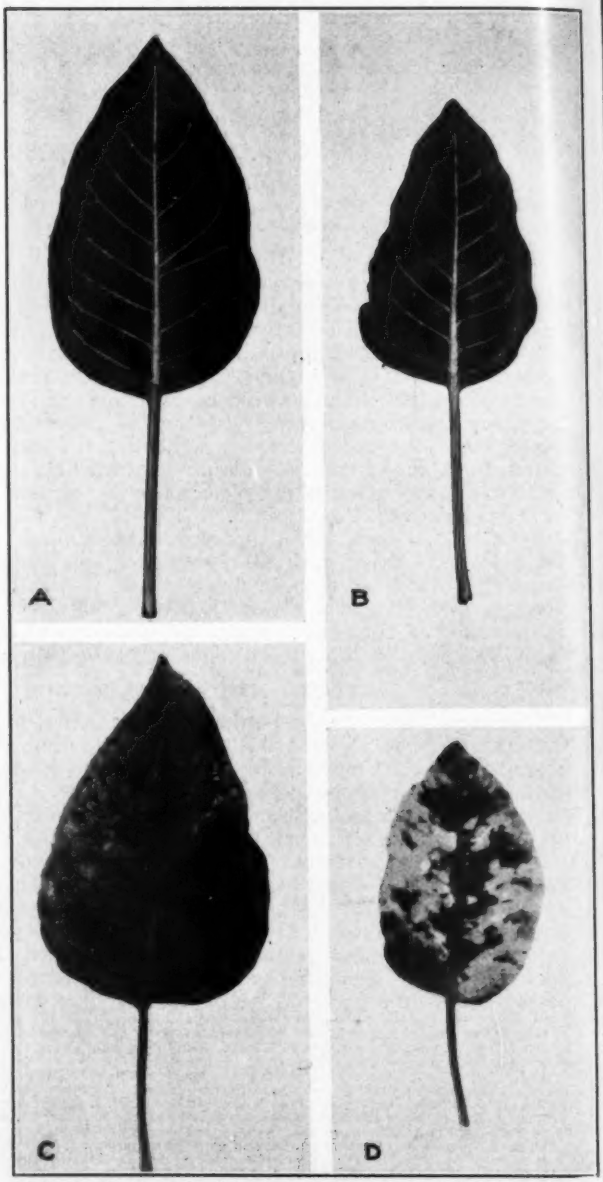

FIGURE 2.—Mosaic on *Nicotiana glauca:* A, Mosaic-free leaf; B, mild mosaic; C, light-green mosaic; D, yellow mosaic. Collected on the island of Grand Canary, Canary Islands. × 1

weed among the potatoes. In one garden at Orotava, fuchias (*Fuchsia magellanica gracilis*) and cultivated peppers (*Capsicum frutescens* L. var. *grossum*) had typical green mosaic. Many tomato plants were examined on the islands, but no mosaic was observed. Also no mosaic was observed on the cucurbit species which were found.

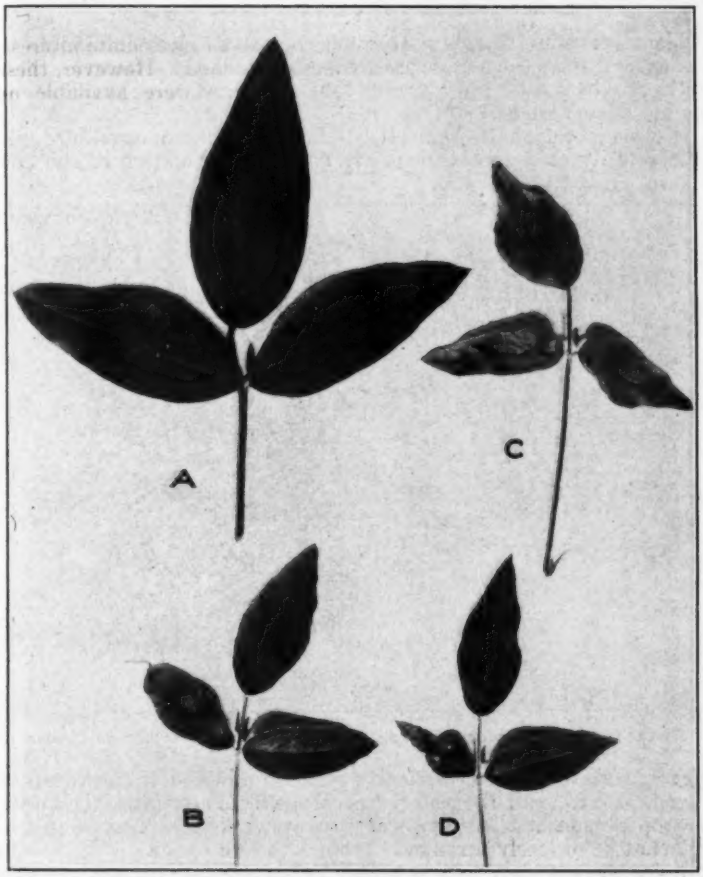

FIGURE 3.—Green mosaic on *Psoralea bituminosa:* A, Healthy leaf; B–D, diseased leaves. Collected on the island of Teneriffe, Canary Islands. × ⅓

Bananas are grown in all suitable areas on both of the islands. Many plantations were visited and hundreds of plants were examined for bunchy top, a destructive virus disease (7) which occurs in Australia, Fiji, Egypt, Ceylon, and the Philippine Islands. None of the plants examined gave evidence of this disease, and no mosaic was observed.

Psoralea bituminosa L., a legume which is a native of Arabia and the Mediterranean districts, and *Nicotiana glauca*,[5] a native of South America, were found growing wild in all the waste places and barrancos or dry creek channels. Both species had green mosaic. (Figs. 2 and 3.) In fact, it was exceptional to find mosaic-free plants of either of the species on Teneriffe or Grand Canary.

EXPERIMENTS WITH THE CANARY ISLANDS MOSAICS

The mosaic on *Nicotiana glauca* was of greatest immediate interest, and several collections of diseased tissue were made. However, these could not be tested until young tobacco plants were available on the expedition yacht.

It was found that Connecticut-Havana tobacco developed very satisfactorily on a special deck arranged near the stern of the boat.

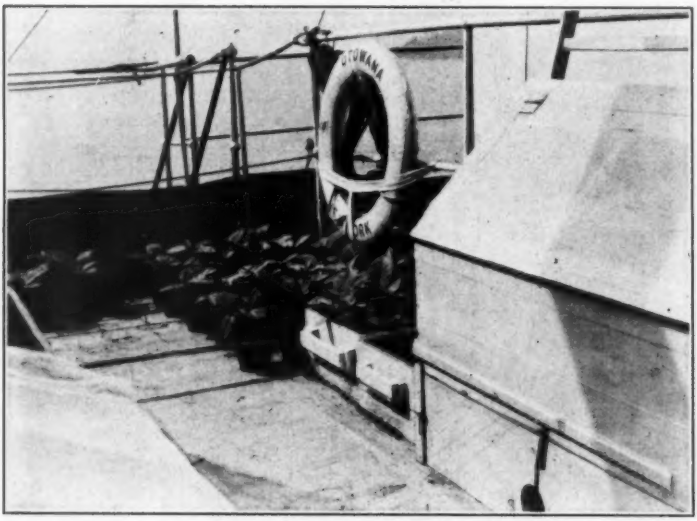

FIGURE 4.—Method of growing experimental tobacco plants on the deck of the yacht *Ulowana*

(Fig. 4.) As no serious difficulty was experienced from storms, it was possible to grow the plants in the open. The intense light from the tropical sun and any salt-water spray were kept from the plants by means of properly arranged cheesecloth and canvas. The plants were grown in 4-inch pots arranged in a large meshed net made of heavy cord and wire. This prevented the pots from rolling about.

An inoculation test was made with an extract from dried leaf tissue obtained from a mosaic-affected plant of *Nicotiana glauca* collected in Teneriffe. This mosaic was easily transmitted to tobacco. From the beginning, the symptoms were of the light-green type. After the plants had developed 12 to 18 leaves, yellow spots and

[5] This species grows wild in many districts in the southwestern part of the United States, and mosaic-diseased specimens have been sent to the writer by David Fairchild from the Boyce Thompson Southwestern Arboretum at Superior, Ariz.

patches appeared on some of the foliage, as indicated in Figure 5, A. These results were of especial interest as they are similar to those that had been obtained with all the mosaic viruses that had been tested on tobacco previously (*4*, *5*).

Another test was carried out with virus from *Nicotiana glauca* collected in Grand Canary. The green-mosaic symptoms were slightly different from those obtained in the previous experiments, but yellow spots occurred in the same manner.

When the ship returned to the Canary Islands for fuel in March, one day was spent collecting and observing mosaic-affected *Nicotiana glauca* growing in the regions of the upper and lower highways between Las Palmas and Telde on the island of Grand Canary. Several hundred diseased plants were examined, and of these one had what

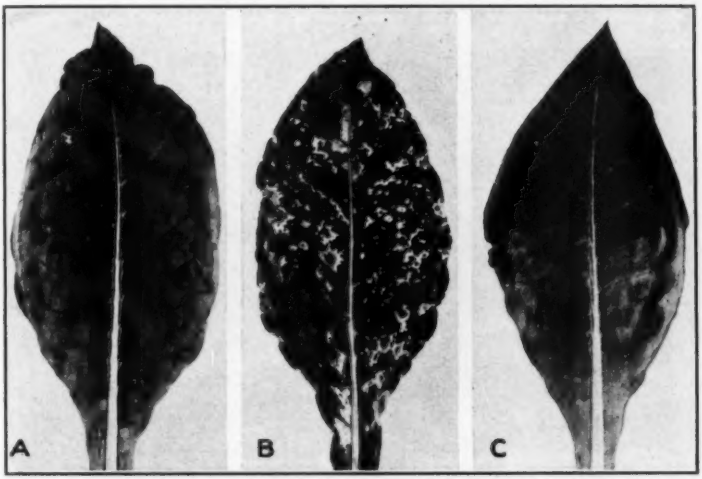

FIGURE 5.—Mosaic on Connecticut-Havana tobacco: A, Light-green mosaic showing a yellow spot; B, yellow mosaic produced by a concentrated virus obtained from spots similar to that shown in A; C, mild dark-green mosaic obtained from *Nicotiana glauca*. × ⅓

appeared to be pure yellow mosaic (fig. 2, D) and two had a yellow and green combination with yellow predominating. The remainder had either a light-green (fig. 2, C) or a mild dark-green mosaic (fig. 2, B). In some cases leaf abnormality was associated with green mosaic.

Mosaic leaf tissue was collected from eight specimens which had somewhat different symptoms ranging from the mild dark-green mosaic to the yellow type. These tissues were handled with great care to prevent cross contamination after collection. The fresh leaves were cut into fine pieces and dried in a warm closet, after which they were carefully stored in envelopes separated from each other by means of heavy oiled paper.

All these dry tissues have been tested on a strain of Connecticut-Havana tobacco in a quarantine greenhouse, and each produced mosaic one year after collection. Ten or more plants were inocu-

lated with each sample. Plants inoculated with virus from different sources were protected from one another by means of frames having galvanized wire screen (12 meshes to the inch each way) tacked on each side, as shown in Figure 6. A frame of single screen was placed in front of the plants. Extensions were arranged on top of the partition screens if the plants became very tall. All openings in the greenhouse were covered with copper screen having 30 wires to the inch each way. The legs of the greenhouse benches were coated with "tanglefoot" paste. The benches did not touch the walls of the house at any point.

Thus far it seems that the mottling symptoms produced by these viruses on tobacco fall into three general classes: (1) Yellow mosaic, a type which has little or no chlorophyll in the lightest-colored areas; (2) light-green mosaic, a type characterized by light-green areas which occupy the greatest portion of the leaf surface, normal dark-

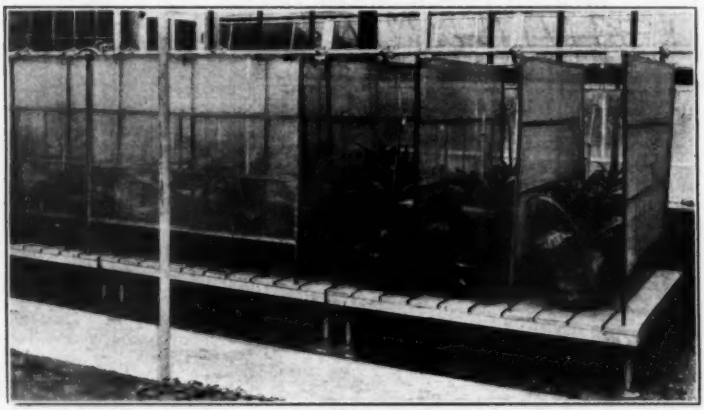

FIGURE 6.—Method used in conducting greenhouse experiments with different viruses. One front screen was removed for the purpose of making the photograph

green areas, and occasional yellow spots; and (3) mild dark-green mosaic, a type in which the dark or normal green is usually in excess of the light-green portion of the leaf, and yellow spots occur rarely. In young plants mild dark-green mosaic is very inconspicuous.

The yellow mosaic, thought to be pure when collected, proved not to be free from the green type. In fact, it appears that the three viruses were present in some of the diseased plants collected. All the green mosaics collected have been studied on maturing tobacco plants, and all have produced yellow spots. Some of the light-green types developed yellow spots in all the inoculated plants. One of the mild dark-green forms has produced yellow spots on 2 of the 52 plants which have been inoculated.

When yellow spots were removed from mosaic-affected leaves and inoculated into young plants, a yellow type of mosaic developed soon after the inoculation. (Fig. 5, B.) By making several successive subinoculations from the increasing number of yellow spots, strains of concentrated virus of yellow mosaic have been developed. From

three to seven isolations and subinoculations have been required to accomplish this. Figure 7 shows a plant inoculated with such a concentrated virus. The yellow mosaics isolated thus far from *Nicotiana glauca* are similar in appearance to the yellow mosaic isolated by the writer (*4*) from cultivated tobacco having light-green mosaic.

Yellow mosaics are very attractive and ornamental, but they are more destructive than the green types. When they are inoculated into tomato plants, a high percentage of killing frequently results. In some cases young tobacco plants have been killed, and in all cases tobacco plants are markedly stunted, and the lower leaves become very yellow and frequently are killed completely.

One of the outstanding characteristics of the viruses of yellow mosaic is their ability to produce a general chlorosis on the old foliage.

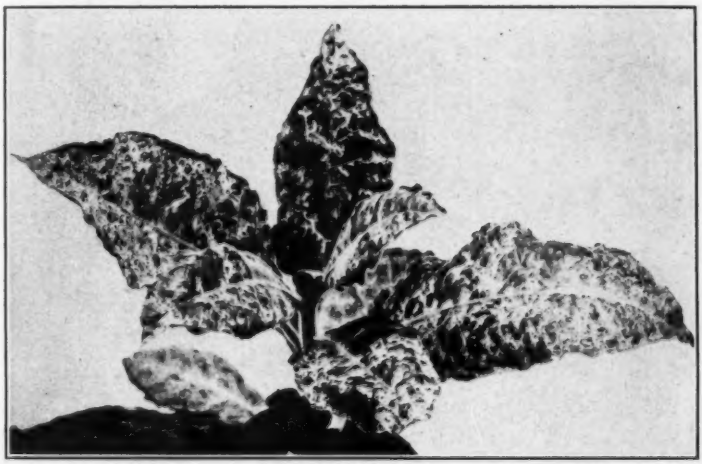

FIGURE 7.—Yellow mosaic on Connecticut-Havana tobacco. The virus used was concentrated by isolating yellow spots from tobacco leaves (fig. 5, A) infected with light-green mosaic induced from virus collected on *Nicotiana glauca*. × ¼

Tobacco plants 30 inches tall at the time of inoculation have developed chlorosis of the type shown in Figure 8 on all the leaves which were over 3 inches in length at the time of inoculation.

The yellow mosaic pattern on the older leaves is quite distinct from that which occurs on the young leaves (fig. 7), and it is very distinct from the yellowing associated with senility. This chlorotic pattern occurs in a milder form on detached leaves which are inoculated with the virus of yellow mosaic and cultured in sand according to the methods of Purdy (*9*). Mottling is very pronounced on the stems and fruit of tobacco plants having yellow mosaic. (Figs. 9 and 10.)

Many combinations of yellow and green mosaic symptoms have been produced by inoculating tobacco and tomato plants with extracts consisting of different proportions of viruses from yellow mosaic and light-green mosaic plants. Several of these synthetic combinations resembled yellow and green mosaics which the writer ob-

served on *Nicotiana glauca* in the Canary Islands, and on tomato plants in the southern United States.

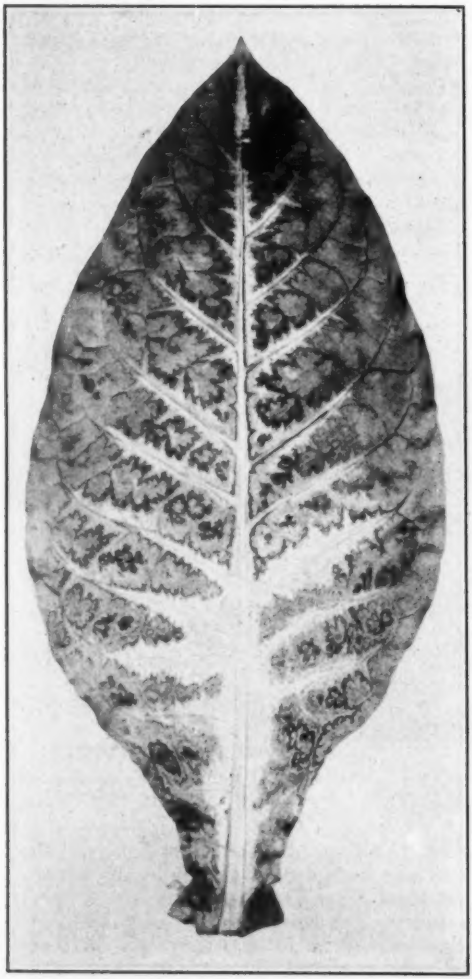

FIGURE 8.—Mature leaf of Connecticut-Havana tobacco. This leaf was about 3 inches long and the plant was 36 inches tall at the time of inoculation with a virus of yellow mosaic. The inoculation was made at the base of the stem. All leaves below the illustrated leaf gradually developed this type of chlorosis and died prematurely. Leaves above this one developed a mottling more nearly like that shown in Figures 5, B, and 7. The dark area near the apex was normal green as compared with leaves of the same stage of development on healthy plants. × ⅜

FIGURE 9.—Yellow mottling on a stem of Connecticut-Havana tobacco, induced by a concentrated virus of yellow mosaic. × 1

The light-green type of mosaic from *Nicotiana glauca* is very similar in appearance, when transferred to tobacco, to the light-green mosaic which occurs on tobacco in the United States. It is

especially like the light-green mosaic which the writer has studied on tobacco (*5*). Differences in the properties of the viruses may exist, however.

The mild dark-green mosaic collected on *Nicotiana glauca* produces very little mottling on tobacco in the early stages of the disease. The first indication of disease consists of a bleaching of the veins. The leaves may become slightly stunted and more or less deformed. Sometimes a very faint green mottling may be noted at this period, but in most cases no mottling is evident. The later leaves show less

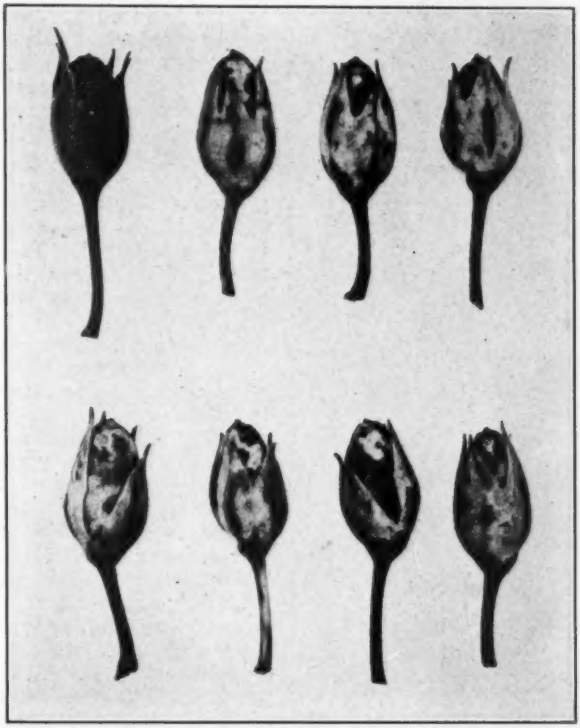

FIGURE 10.—Yellow mottling on the calyx and fruit of Connecticut-Havana tobacco, induced by a concentrated virus of yellow mosaic. Mosaic-free specimen at upper left. × 1

deformity. They are normal green in color, and no mottling can be detected. From three to five such leaves may develop. These are followed by three to five leaves which show very small, faint light-green mottlings as shown in Figure 11. This condition becomes more intense on subsequent leaves until a very pronounced mottling occurs. This mottling is characterized by very large light and dark green areas (fig. 5, C), and sometimes more than half of the leaf is solid dark green and the remainder solid light green. The margins of the leaves frequently turn downward, causing an inverted spoon effect as shown in Figure 12. This stage may be followed by one in

which a few leaves develop very small, faint, light and dark green mottlings as in the case of the earlier stage. These observations are based on more than 50 plants which grew to maturity.

FIGURE 11.—Mild or faint mottling on Connecticut-Havana tobacco leaf, induced by a virus of mild dark-green mosaic collected on *Nicotiana glauca* in the Canary Islands. This mottling occurs in conjunction with the type illustrated in Figure 5, C. × ⅔

Thus far the mild dark-green mosaics have shown much less evidence of the yellow type than have the light-green forms, and at present it appears that the mild green types offer the best opportunity for obtaining a green mosaic free from yellow mosaic.

The writer has isolated a mild dark-green form from a slightly mixed infection occurring in *Nicotiana glauca* collected on Grand Canary, and this virus seems to be free from viruses causing the yellow and the light-green types of mosaic.

Inoculations have failed to transmit the mild dark-green mosaic of *Nicotiana glauca* to tomato plants grown from Canary Island seed as well as from American seed. The light-green and the yellow mosaics from *N. glauca* have been transmitted to tomatoes. Some of the tobacco mosaics of American origin have failed to produce symptoms in *N. glauca* collected on Teneriffe, but in some cases *N. glauca* has proved to be a carrier of these viruses after being inoculated. These same mosaics were transmitted by Johnson (*2*) to *N. glauca* from Italy, and symptoms were produced. From correspondence with Roy E. Clausen it is evident that all collections of *N. glauca* are not alike genetically. It appears, therefore, that the successful use of wild and domesticated hosts for differentiating viruses depends on the use of strains known to be homozygous for resistance or for susceptibility to a given mosaic. Obviously, workers in this field can not accurately check one another's results until a stock of pure-line seeds is available.

When tobacco plants with mild dark-green mosaic were reinoculated with the virus of one of the writer's yellow mosaics, the

former type became obscured and the plants showed the typical symptoms of yellow mosaic. When plants with one of the writer's light-green mosaics were reinoculated with the same virus of yellow mosaic no change in the symptoms occurred. The plants continued to produce typical light-green mosaic after three reinoculations with the virus of yellow mosaic.

Leaf deformity is very frequently associated with mosaic on tobacco and *Nicotiana glauca*. Some of these deformities (fig. 12) occur with

FIGURE 12.—Extreme leaf deformations produced on Connecticut-Havana tobacco inoculated with a virus obtained from mosaic-diseased *Nicotiana glauca* collected at Gibraltar. One midrib had no lamina and another possessed only a small portion of a lamina. × ½

considerable regularity regardless of special environmental conditions, whereas others (fig. 13, A) occur only under certain limited conditions and do not continue throughout the life of the plant.

Some of the long narrow leaves shown in Figure 13, A, have distorted vascular elements. The veins are fused in many cases. Also the lamina is often distorted on the underside of the leaf forming liplike structures as shown in Figure 13, B. These leaf deformities are seemingly accentuated by reduced light, and they occur most frequently

in tobacco plants which are inoculated when very small. Woods (*13*) found that mosaic-affected tobacco produced deformed leaves after the plants had been cut back. The writer has obtained the same results with mosaic-affected tobacco plants and also with

FIGURE 13.—Mosaic on Connecticut-Havana tobacco: A, Deformed leaves of tobacco produced by a light-green mosaic virus obtained from domestic tobacco. This plant was grown in shade during its early development after inoculation. This deformity does not continue as the plants develop, especially if they are given full sunlight. × ½. B, A portion of the underside of a deformed leaf showing the liplike structure in the lamina and the fusion of laterals with the midrib. × 2

healthy plants. However, in both instances the leaf deformities did not continue throughout the life of the plants.

Other species of mosaic-free Solanaceae behave in the same manner. This phenomenon was observed very commonly in slashings of solanaceous perennials in the bush and the mountain jungles in Africa.

MOSAICS IN WEST AFRICA

The expedition reached Bathurst, capital of Gambia, early in January. Almost immediately a trip was made up the Gambia River to McCarthy's Island, which is located in the savanna type of vegetation.

In a garden located in the native village of Sankule, near Georgetown, mosaic was found on tabasco pepper and on eggplants. It was found again on cultivated eggplants in Monrovia, Liberia.

Colonial agricultural workers informed the writer that the rosette disease is very destructive on peanuts (*Arachis hypogaea* L.) in Gambia and in Sierra Leone. In some seasons this disease is the limiting factor in peanut production. The peanut crop had been harvested, and therefore it was not possible to observe rosette. This virus disease causes considerable damage in several regions in East, West, and South Africa (*12*). It has not yet been reported in the United States.

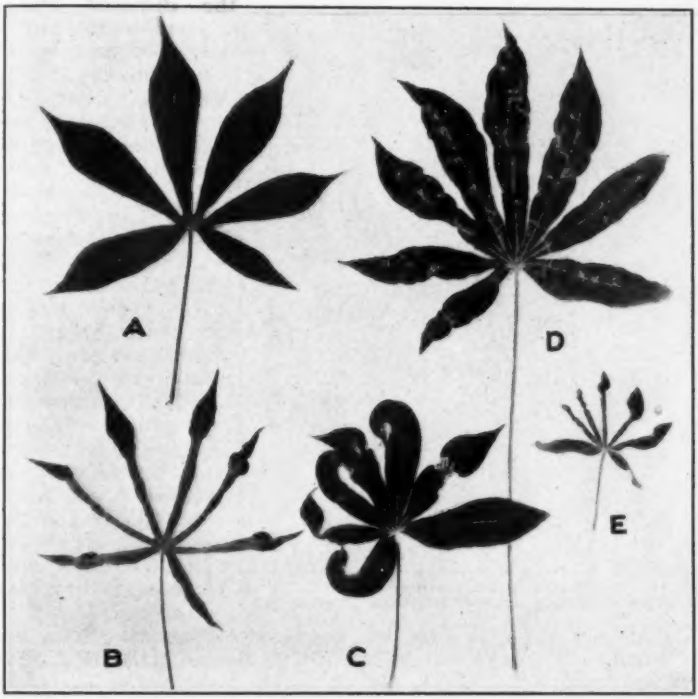

FIGURE 14.—Light-green mosaic on leaves of cassava (*Manihot* sp.): A, Healthy leaf; B–E, diseased leaves. Collected in Sierra Leone. × 1/4

In all the colonies visited, from Liberia to French Cameroon, mosaic was prevalent on Cayenne, chili, and tabasco types of pepper (*Capsicum frutescens* L.) and on several types of cassava (*Manihot* sp.).

The mosaic on pepper is of the green type. It is identical in appearance with the mosaic commonly found on peppers in the United States, and it has been transmitted successfully by the writer to healthy chili pepper plants. Doubtless this pepper was taken to the West Coast in the days of the slave trade with the West Indies and South America.

The mosaic occurring on cassava is a yellowish green type and produces a considerable number of deformed leaves, as shown in Figure 14. Although this disease has not been studied, it has the appearance of an infectious mosaic, and it is considered as such by agricultural workers who are familiar with it. In many localities the diseased plants were very much stunted, and the crop undoubtedly was reduced as a result.

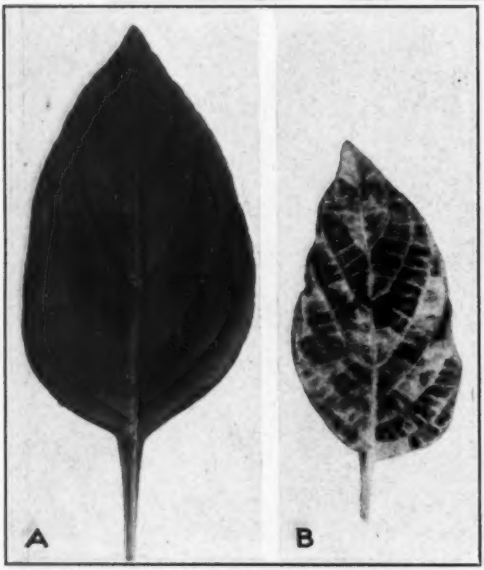

FIGURE 15.—Yellow mosaic on *Asystasia coromandeliana:* A, Healthy leaf; B, diseased leaf. Collected near Buea, British Cameroon. × 1

The tuberous roots of the cassava supply much of the starchy food of the inhabitants of the Tropics of both hemispheres, and the tapioca of commerce is made from the starch derived from this plant. It seems apparent, therefore, that this disease should be studied intensively. Several slightly different kinds of cassava were observed in west Africa, and it is possible that some of these may offer an opportunity for making resistant selections.

The disease was noticeably less abundant on the lower elevations of Cameroon Mountain in the vicinity of Buea. A larger number of mosaic-free pepper plants also were found there than elsewhere. The district is somewhat cooler than the others visited, and it is possible that this has some bearing on the reduced amount of mosaic.

In Duala, French Cameroon, a yellow mosaic was found on *Asystasia coromandeliana* Nees (fig. 15), a weed which is rather abundant in that district. *Fleurya podocarpa* Wedd., a nettle, found on a jungle roadside near Buea, British Cameroon, had yellow mosaic.

The first evidence of mosaic on a cucurbit was found below Buea on Cameroon Mountain. A typical light-green type was found on *Physedra barteri* Cogn., a native wild species. (Fig. 16.) This mosaic resembled the one which occurs on the commercial cucumber

in the United States. The leaves on the mosaic-free plants were much larger than the diseased leaves.

Yellow mosaic (fig. 17) was found on a native cucurbit (*Momordica charantia* Linn.) at the Aburi Agricultural Experiment Station, Gold Coast, and on *Kedrostis foetidissima* Cogn. (fig. 18), a native cucurbit growing in a "bush island" (fig. 19) on the Winneba Plain in the Gold Coast. Plants of *Hibiscus calycinus* (fig. 20) growing 20 miles north of Accra, Gold Coast, also were found to have a yellow mosaic.

Many plants of the calabash gourd (*Lagenaria leucantha* Rusby), which were cultivated by the natives, were examined in all the colonies visited, but no suggestion of mosaic were found on this group of varieties.

The pepper and the eggplant were the only solanaceous plants that were found affected with mosaic in west Africa. Tomatoes cultivated near Buea, British Cameroon, were free from mosaic. In Gambia a few tomatoes of the cherry type, found in a native garden, had what appeared to be a fern-leaf type of disorder. Attempts to transmit this condition to tobacco failed.

FIGURE 16.—Green mosaic on *Physedra barteri*. Collected near Buea, British Cameroon. × 2/3

Many wild species of Solanaceae were found in the Futa Jallon highlands, French Guinea, and on the lower elevations of Cameroon Mountain, but none showed mosaic.

Bananas and plantains were growing in many of the colonies visited. When possible, the plants were examined for indications of mosaic and bunchy top, but there was no evidence of these diseases.

Wild grasses were growing abundantly in the British and French Cameroons, in Fernando Po, and in parts of the Gold Coast. However, many observations failed to reveal any suggestion of mosaic on a grass.

Several types of sugar cane were found in all the colonies from the Gambia to the Cameroons. For the most part these plants were in native gardens. Some patches were found on the agricultural stations and farms, and one large planting was found near Cape Coast in the Gold Coast. Cane plants were carefully examined in all the localities, but no mosaic could be found. Infection experiments show that cane

plants, from stalks collected in west Africa, are susceptible to the cane mosaic prevalent in Louisiana.

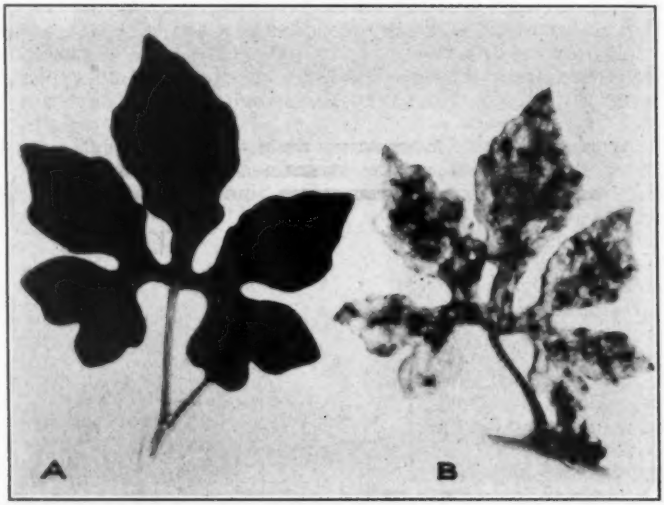

FIGURE 17.—Yellow mosaic on *Momordica charantia:* A, Healthy leaf; B, diseased leaf. Collected at Aburi, Gold Coast. × 1

FIGURE 18.—Yellow mosaic on *Kedrostis foetidissima.* Collected in a "bush island" on the Winneba Plain, Gold Coast. × 1

While in the Gold Coast the writer was informed by the Colonial mycologist that the streak disease occurs on maize in that colony. The disease could not be observed, as the crop had been harvested

some time previously. This is a destructive virus disease which occurs on the East Coast of Africa, in Egypt, and also in South Africa. It has not yet been reported from the Western Hemisphere.

From the literature and verbal descriptions obtained from workers who are familiar with streak, it appears to produce a stripe or streak which is similar in many particulars to the yellow mosaic associated with the green mosaic of wheat and other small grains. This yellow mosaic was mentioned and one type was illustrated by the writer several years ago (*3*). From the illustrations published by Storey (*11*), maize streak also resembles the Cuban maize stripe described by Stahl (*10*).

It is possible that Stahl considers streak and stripe distinct, because he could not transmit stripe to sugar cane, and because Storey failed

FIGURE 19.—Typical "bush islands" in the Winneba Plain, Gold Coast

to transmit streak with *Peregrinus maidis* Ashm., the vector for stripe. Illustrations of the two diseases are so similar that one is tempted to raise the question as to their possible identity in spite of vector differences. On the basis of published information it seems equally plausible that streak and stripe are identical and that *Peregrinus maidis* has biologic forms or races differing in their host preferences or in their ability to transmit a given virus. Certainly there is ample opportunity for such strain differences to manifest themselves in regions so widely separated as Africa and Cuba.

There is no information on the problem of biologic forms within species of insects serving as plant-disease vectors, and it is believed that this problem must be thoroughly explored in order that there may be a better understanding concerning vector relationships and possible differences among certain viruses.

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EXPERIMENTS WITH A MOSAICLIKE DISEASE OF PEANUTS

When the writer was in Sierra Leone, E. Hargreaves, the colonial entomologist, called attention to a so-called mosaic on a few peanut plants he was carrying through the dry period in his compound at the agricultural experiment station at Njala, located more than 100 miles inland from Freetown. The writer took parts of diseased and healthy plants back to the yacht for inoculum and for obtaining photographic records. (Fig. 21.)

FIGURE 20—Yellow mosaic on leaf of *Hibiscus calycinus*. Collected near Accra, Gold Coast. × 1

The tissue was placed in the ship's cool room until young peanut plants were available for inoculation. Plants were ready for inoculation in a few days, and at this time it was found that many aphids had been increasing on the diseased tissue. These were carefully removed to several of the young healthy plants. In from four to five days after this transfer was made the new leaves on the aphid-infested plants showed mottling identical with that on the original material. The control plants, which had no aphids, were free from mottling. As only one lot of aphids was available it was not possible to determine the effects from insects which had fed on healthy plants. However, it was considered that in case the mottling were an infectious mosaic it would persist after the removal of the aphids. If it were the result of aphid attack, the plants would recover after the removal of the insects.

The aphids were removed from part of the mottled plants by means of a soapsuds spray. On these plants the new foliage was free from mottling, whereas the mottling continued on the new leaves of the plants which still harbored aphids. The mottlings developed again on the new foliage of recovered plants when aphids were allowed to feed on them again. The control plants which were kept free from aphids remained healthy.

The mottling differed somewhat from that of the ordinary types of green mosaic, and, from the behavior of the disease, it seems likely

that it is not a mosaic of the virus type. It seems to behave more nearly like some of the diseases of forage legumes which presumably are produced by the toxic products of certain species of leaf hoppers (*8*).

Unfortunately, the aphids were lost before they were identified, and further effort is necessary before identification is possible.

MOSAIC IN GIBRALTAR

On returning to Gibraltar late in March, the writer spent several days collecting. On one of the collecting trips a plant of *Nicotiana glauca* was found growing from a crevice in the side of the rock facing the Mediterranean Sea near Europa Point. This plant was just within reach, and on examination it was found to have a mild form of green mosaic. The virus from the dried leaves of this plant produced mosaic in tobacco plants one year after the collection was made. This mosaic has been studied only in a preliminary way. It appears to be very similar to the mild dark-green types collected

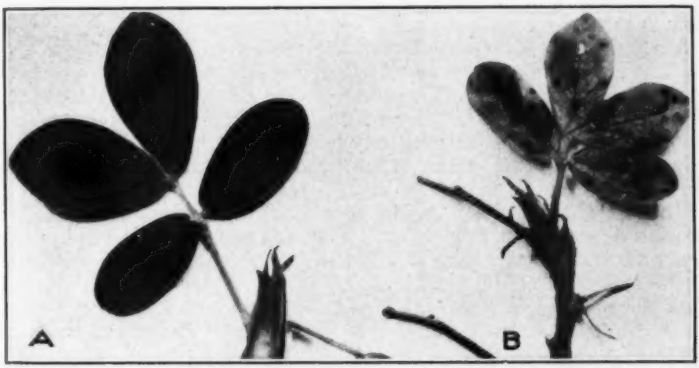

FIGURE 21.—Leaves of peanut (*Arachis hypogaea*): A, Healthy; B, mosaiclike mottling caused by aphids. Collected in Sierra Leone. × 1/10

in the Canary Islands. It frequently produces extreme leaf deformity, as shown in Figure 12.

DISCUSSION

The writer recognizes the importance of making inoculation tests before concluding definitely that a chlorotic condition is an infectious mosaic. It has not been possible to make inoculation tests with all of the collections, and it is possible that some of the mosaics described are not caused by viruses. However, in all the cases described, the symptoms were very typical for green or for yellow types of mosaic.

Mosaics of *Nicotiana glauca* obtained on Grand Canary, Teneriffe, and Gibraltar are of considerable experimental interest.

The collections from the Canary Islands have been studied more thoroughly than the others, and it has been found that each mosaic-diseased specimen was affected by more than one type of mosaic. In all cases a yellow mosaic is associated to a greater or less extent with the green type, and in some cases it appears that two green mosaics and the yellow type are associated in the same plant.

Evidence obtained thus far indicates that the yellow mosaic is associated more generally with the light-green mosaics, and at the present time the mild dark-green types seem to offer an opportunity for obtaining a single virus of green mosaic.

It is of especial interest that each of the 17 viruses of light-green mosaic which have been tested on tobacco, and in some cases on tomato, contains virus of yellow mosaic. These viruses were obtained from seven different States in the United States and from Hawaii, England, and the Canary Islands.

If these associations of green and yellow mosaic, which appear to be rather general, are due to simple admixtures of two independent types or strains of virus, it would seem that there would be more evidence of a relatively pure yellow mosaic in fields of tobacco and tomatoes and in wild *Nicotiana glauca*. The writer has observed yellow mosaic most frequently in southern-grown tomatoes, but even in this case the number of plants has been exceedingly small in comparison with the large number in which green mosaic predominated. The fact that a yellow mosaic was not reported on field tobacco until recently by Johnson (*2*) is good evidence that this form is much less general than the green types.

A satisfactory interpretation of the yellow and green mosaic associations can not be given at this time because so few data are available. It is possible that these are a simple admixture of independent viruses comparable to a mixed infection of two or more species of fungi. However, it seems entirely possible that viruses may become altered locally in the plant, thus producing mutations, to use this term in its broadest meaning.

No attempt has been made to classify the viruses causing the mosaics described in this paper. This can not be done until studies now under way have been advanced. Any method of classification depends largely on the purity of the viruses under study. Johnson's work shows that differential hosts, heat, and chemical treatments assist greatly in separating types of viruses. However, there are instances in which these methods have not yet proved effective, as in the case of the association of the yellow and green mosaics described previously (*4*).

The writer's work shows that the studies on virus purification and classification should not be confined to young plants. In many cases plants must be carried to maturity in order to determine mixtures of viruses and to obtain a full knowledge of the symptom produced by a given pure virus.

It appears also that classification work should be based on host material which is homozygous for resistance or susceptibility to the mosaics being tested. Also, attention should be directed to the influence of toxic or other plant products on the behavior of a given virus after it has been passed through several species of hosts. The importance of homozygous host plants has been strikingly evident in the work on wheat mosaic. In the case of tobacco, the writer obtains seed from a single plant of known history and propagates it vegetatively in the greenhouse under conditions which prevent its receiving pollen from other plants.

It is not known how general the association of yellow and green types may be among the mosaics of other groups of plants. This association was observed in winter wheat (*3*) in 1920 near Granite

City, Ill., and it has been evident in experimental plot tests conducted since that time. Experiments show that yellow mosaic occurs more frequently in certain species of small grains and in certain varieties within a given species. Further study is required to determine whether the yellow and green mosaic association in the small grains is in any way analogous to the one occurring in the Solanaceae. Results obtained thus far seem to indicate that the symptoms of mosaic types in the small grains are influenced in part by genetic factors in the hosts.

Yellow mosaics are more devastating than the green types that have been observed, and it is fortunate that they are rare.

SUMMARY

In the Canary Islands green mosaics were found on *Solanum tuberosum*, *Nothoscordum fragrans*, *Capsicum frutescens* var. *grossum*, *Fuchsia magellanica gracilis*, *Psoralea bituminosa*, and *Nicotiana glauca*. Yellow mosaic was found on three plants of *N. glauca*.

Experimental studies have been carried out with Connecticut-Havana tobacco inoculated with viruses from the 10 mosaics collected on *Nicotiana glauca* in the Canary Islands. The results of these tests show that the green forms of mosaic are of two types, a light green and a mild dark green. The green forms that have been studied thoroughly were found to contain, to a greater or less degree, traces of a yellow mosaic virus which can be concentrated by successive spot isolations and subinoculations. The light-green mosaics develop more yellow mosaic spots than do the mild dark-green forms, and it appears that a strain of the latter has been isolated which is free from viruses of the yellow and the light-green mosaics.

In west Africa green mosaics were found on eggplant, pepper, *Manihot* sp., and *Physedra barteri*. Yellow mosaics were observed on *Fleurya podocarpa*, *Asystasia coromandeliana*, *Momordica charantia*, *Kedrostis foetidissima*, and *Hibiscus calycinus*.

The rosette disease occurs on peanuts in Gambia and Sierra Leone, and maize streak occurs in the Gold Coast.

In Sierra Leone a mosaiclike mottling occurs on peanuts. This is produced by aphids and seems not to be caused by a virus.

In Gibraltar mild dark-green mosaic was found on *Nicotiana glauca*.

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STUDIES OF FIRE BLIGHT OF APPLE IN WISCONSIN[1]

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INTRODUCTION

Fire blight, caused by *Bacillus amylovorus* (Burr.) Trev., is one of the major diseases of pomaceous fruits. The destructiveness and widespread occurrence of this disease have contributed toward making it the subject of numerous investigations. Inasmuch as most of the voluminous literature which has resulted has been well reviewed by Jones (*17*)[3], Stewart (*28*), Brooks (*5*), and others a general review of literature appears to be unwarranted. Such discussions of previous work as seem necessary appear in the body of the paper under appropriate headings.

Fire blight varies greatly both in severity of occurrence and in difficulty of control. In Wisconsin, epidemic outbreaks are frequent and destructive. Numerous attempts of the apple growers of this State to control the disease by the commonly recommended methods have been attended with but indifferent success. This variability in the severity of occurrence of fire blight and the difficulty of its control in epidemic years have led to further studies of the disease under local conditions. In these studies it seemed advisable to seek first a more adequate understanding of the course of disease development in relation to variability. Two correlated lines of study were undertaken: (1) Studies of the development and control of the disease in relation to the natural environment and (2) detailed studies under partly controlled conditions in the laboratory and greenhouse of certain phases of the life history of the causal organism in relation to pathogenesis.

DEVELOPMENT OF THE DISEASE IN RELATION TO THE NATURAL ENVIRONMENT [4]

SEASONAL DEVELOPMENT RECORDS

Blossom development of five of the more important apple varieties grown at Gays Mills, Wis., was followed from the time the cluster buds began to open until petal fall. From the data gathered the blooming periods of each of the varieties under observation were determined. These data are expressed graphically in Figures 1 to 3. Twig development was followed by tagging and measuring at frequent intervals 20 terminal twigs, representatively distributed, on

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[2] The writer wishes to express his indebtedness to Dr. G. W. Keitt, under whose direction this work was done, for suggesting the importance of the problem and for helpful suggestions and constructive criticisms during the progress of the investigation and the preparation of the manuscript. Grateful acknowledgments are also made to Prof. L. R. Jones and Dr. A. J. Riker for helpful advice, and to the stockholders and manager of the Kickapoo Development Company of Gays Mills, Wis., for their cordial cooperation in making the field studies possible.

[3] Reference is made by number (italic) to "Literature cited," p. 620.

[4] Studies of the development of the disease in relation to the natural environment were conducted in commercial orchards at Gays Mills, Wis. For three growing seasons (1926–1928) the writer lived in a tent laboratory in the orchards, thereby gaining an excellent opportunity for daily observations and records.

each a resistant and on a susceptible variety. From the data obtained the average length of the current year's growth in inches was computed. (Figs. 1–3.)

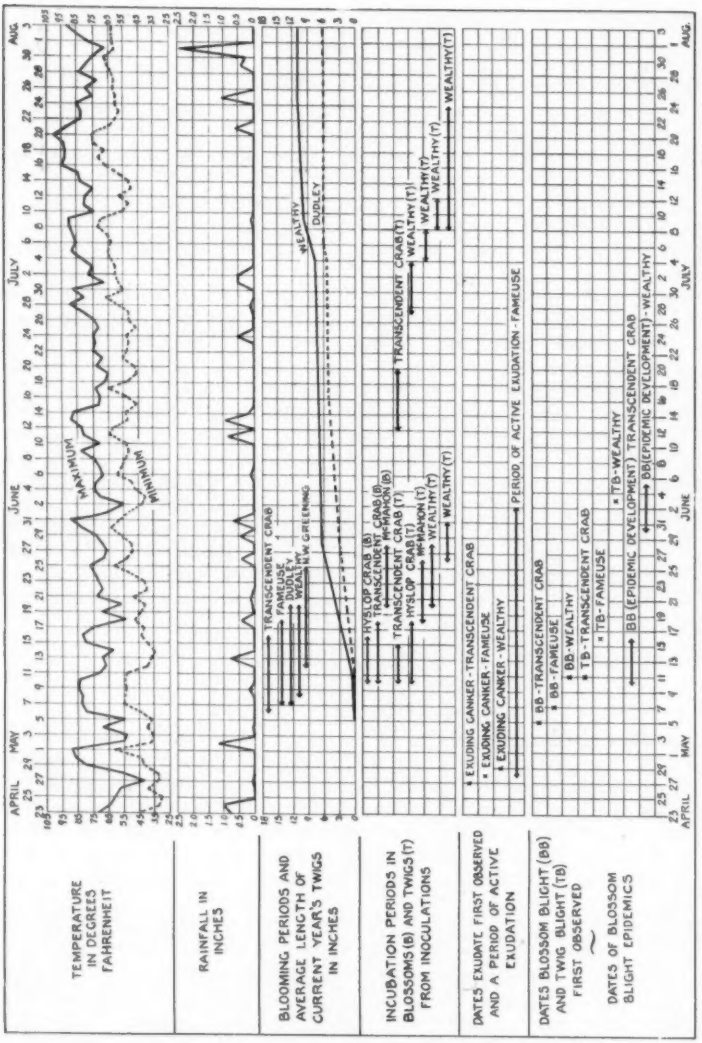

FIGURE 1.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gays Mills, Wis., and observed April 23 to August 3, 1926

A number of cankers which appeared likely to be active were tagged before any bacterial exudate was apparent. These cankers were examined daily, and the dates on which they started to exude and the subsequent dates of exudation were noted. Tags were placed

on 500 blossom clusters and 150 twigs representatively distributed on a number of Fameuse trees, and daily visits were made to each cluster and twig to determine whether blighting had taken place.

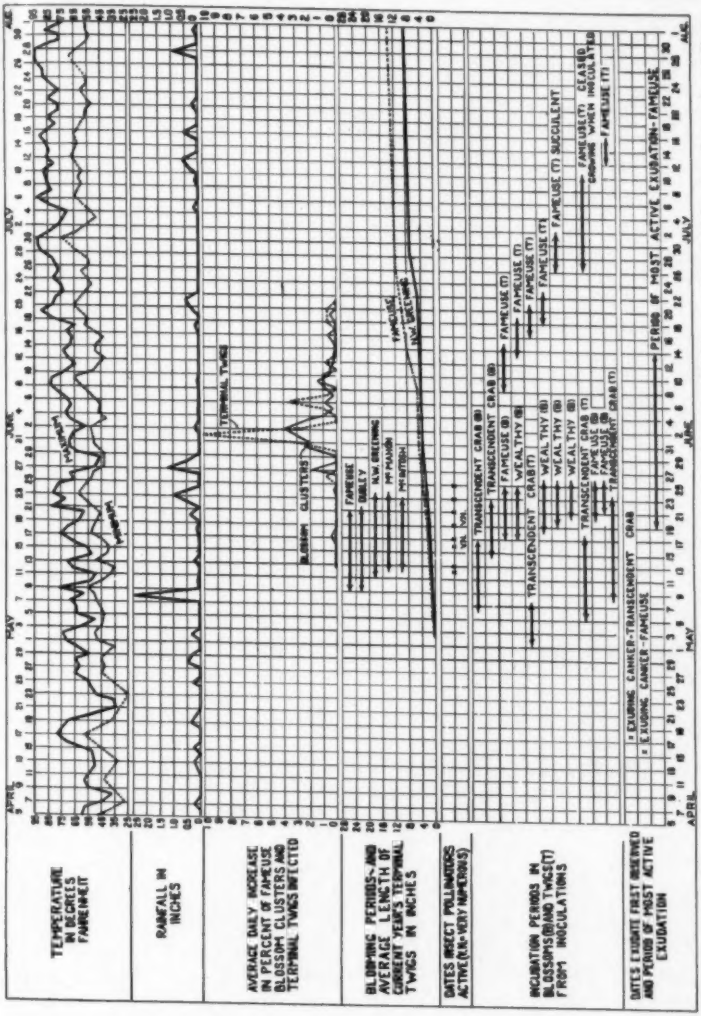
TEMPERATURE IN DEGREES FAHRENHEIT
RAINFALL IN INCHES
AVERAGE DAILY INCREASE IN PERCENT OF FAMEUSE BLOSSOM CLUSTERS AND TERMINAL TWIGS INFECTED
BLOSSOM CLUSTERS
TERMINAL TWIGS
BLIGHTING PERIODS AND AVERAGE LENGTH OF CURRENT YEAR'S TERMINAL TWIGS IN INCHES
DATES INSECT POLLINATORS ACTIVE (NA=VERY NUMEROUS)
INCUBATION PERIODS IN BLOSSOMS AND TWIGS (T) FROM INOCULATIONS
DATES EXUDATE FIRST OBSERVED AND PERIOD OF MOST ACTIVE EXUDATION
EXUDING CANKER-TRANSCENDENT CRAB
EXUDING CANKER-FAMEUSE
PERIOD OF MOST ACTIVE EXUDATION-FAMEUSE
APRIL
MAY
JUNE
JULY
AUG.

FIGURE 2.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gays Mills, Wis., and observed April 5 to August 1, 1927

The results of these studies are presented graphically in Figures 1 to 3 in terms of the average daily increase in the percentage of terminal twigs and blossom clusters blighted.

Studies of the development of the disease under natural conditions were supplemented by artificial inoculation experiments. Unopened blossoms in the green-tip and early closed-cluster stages of development, and succulent twigs were inoculated from time to time by making wounds in the tissues with sterile needles and atomizing pure

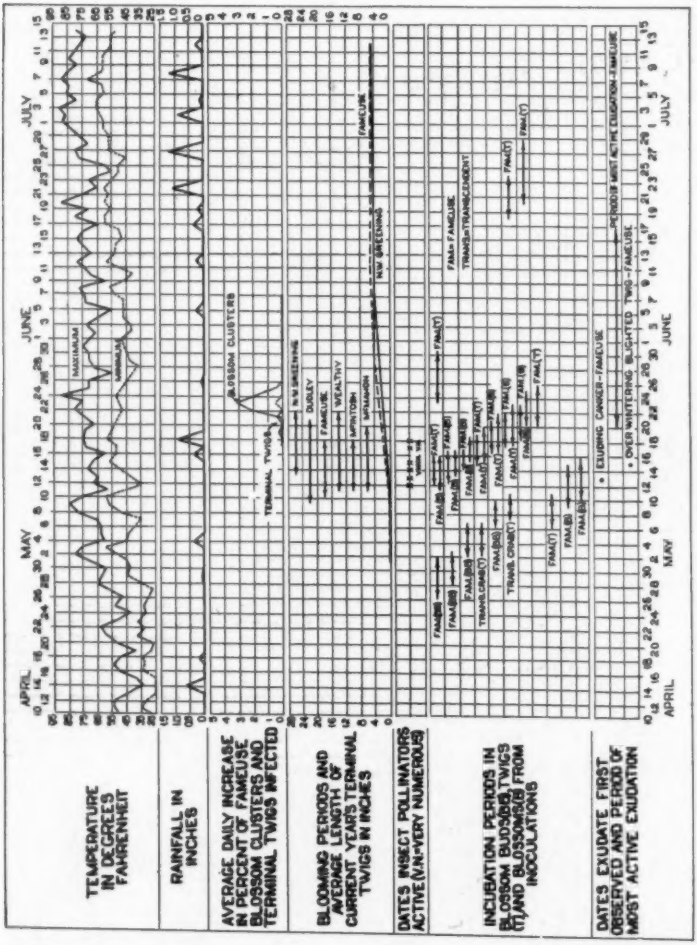

FIGURE 3.—Graphic summary of certain records pertaining to the epidemiology of fire blight on certain varieties of apple growing at Gays Mills, Wis., and observed April 10 to July 15, 1928

nutrient broth cultures of *Bacillus amylovorus* upon the wounded surfaces. Open flowers were inoculated at intervals starting from the time the blossoms first opened and continuing until several days after petal fall. These inoculations were made by spraying pure broth cultures of *B. amylovorus* into the receptacle cups with an atomizer. Before and after inoculation the unopened and open

blossoms were protected from natural infection by bagging. Daily visits were made to the inoculated blossoms and twigs and the incubation periods determined. The incubation periods as shown by these experiments appear in Figures 1 to 3. It is recognized that the data gathered in these studies on seasonal development are to be interpreted as approximately representative rather than as quantitatively exact.

METEOROLOGICAL RECORDS

The relative humidity and air temperature were recorded by means of a hygrothermograph which was housed in a standard instrument shelter set about 4 feet above the ground. The instrument was adjusted at frequent intervals by means of a sling psychrometer and a standardized thermometer.

Records of rainfall were taken by means of a standard rain and snow gauge.

DISCUSSION OF RESULTS

Yearly graphic summaries of disease development and meteorological data appear in Figures 1 to 3. While a more detailed analysis of these data will be presented later in relation to the various topics to which they are pertinent, it may be noted here that natural infection periods in both blossoms and twigs appear to be closely correlated with rain periods. Symptoms of the disease commonly became evident after a normal incubation period following rains, as was revealed by the inoculation experiments reported. The fact that twig blight begins to become manifest at approximately the same time as blossom blight is of striking significance in relation to the mode of dissemination of the natural inoculum. (See p. 588.)

FIELD STUDIES OF THE DEVELOPMENT OF EPIDEMICS

Fire-blight epidemics in Wisconsin are frequent and often very destructive. In some years the disease may be mild in its attack in one locality and severe in others. A knowledge of the chief factors concerned in the development of blight epidemics is obviously of great importance in relation to the control of this disease. It is sought in the following pages to trace the development of epidemics and to define critical periods in their occurrence and control.

MODES OF OVERWINTERING OF THE CAUSAL ORGANISM

Hold-over Cankers

The work of Waite (*31*), Whetzel (*34*), Jones (*17*), Brooks (*5*), and others has called attention to the fact that *Bacillus amylovorus* lives through the winter in the different hosts which it attacks largely in association with certain cankers which are known as "hold-overs." Only a comparatively few of the cankers formed in any one year, however, overwinter the causal organism, as most of the cankers are inactivated soon after their formation. Brooks (*5*) reports that 8 per cent of the cankers examined in Fameuse apple trees in 1925 were found to be hold-over sources of primary inoculum. The present writer found that 6 per cent of the cankers examined in Fameuse apple trees in 1926 overwintered the bacteria and in 1927, 7 per cent of those examined in this variety were hold-overs. In this work the extension

of the margins of the cankers in the spring and the production of exudate from the surface of the discolored areas have served to indicate those cankers which overwintered the causal organism. Only 3 per cent of the cankers examined in Fameuse trees in 1928 overwintered the bacteria. This scarcity of overwintering sources of primary inoculum may explain, in part, the sparse development of blight in 1928. While the percentage of cankers which overwinter the causal organism in cultivated apple trees is relatively small, it is usually sufficient, under Wisconsin conditions, to furnish a plentiful source of inoculum for primary infection in moderately or heavily infected orchards of the varieties which favor overwintering.

RELATION OF VARIETY OF APPLE

There is a marked difference in the amount of overwintering which commonly occurs in the various commercial apple varieties grown in Wisconsin. In the course of certain studies in 1924 and 1925 Brooks (*5*) observed this variation and states that "of 24 varieties of apple observed, the following were found to overwinter the organism and are listed in the order of their importance in this respect in the situations studied: Transcendent, Hyslop, Yellow Transparent, McMahon, Wealthy, Fameuse, Tolman Sweet, McIntosh, and Dudley." While the present writer has made no attempt to classify all the varieties grown in Wisconsin in respect to their importance in overwintering the fire-blight organism, marked differences in this regard have been noted during the course of these studies. In each year that this disease has been studied by the writer the Fameuse, Transcendent, Yellow Transparent, and Wealthy varieties were found to bear a much larger percentage of hold-over cankers than the McIntosh, Dudley, or Northwestern Greening. The Fameuse and Transcendent varieties were found to be particularly serious sources of primary incoculum as they contained the largest percentage of hold-over cankers in the varieties studied. (Table 1.) Such variations

TABLE 1.—*The per cent of cankers in certain apple varieties found to overwinter Bacillus amylovorus, Gays Mills, Wis., 1926–1928*

Year and variety	Cankers examined (number)	Cankers active	
		Number	*Per cent*
1926			
Fameuse	265	15	6
Wealthy	328	8	2
1927			
Fameuse	2,200	146	7
Wealthy	522	9	2
Dudley	375	1	0
McIntosh	13	0	0
1928			
Fameuse	1,483	38	3
Wealthy	310	2	1
Dudley	310	1	0

in the amount of overwintering which commonly occur in apple varieties may explain, in part at least, the failure of certain investigators to find overwintering sources of primary inoculum in certain localities. It is obviously difficult to find hold-over sources of primary inoculum in varieties which do not commonly overwinter the pathogene.

RELATION OF TYPE OF CANKER

An extensive examination of numerous cankers in 1926, 1927, and 1928 revealed an apparent relationship between the type of canker and its hold-over potentialities. For the most part those cankers which showed no line of delimitation about the periphery of the discolored areas in the fall were the ones most likely to carry the blight organism over the winter. In this type of canker the brownish, discolored portion merges into the apparently healthy tissues. (Fig. 4.) Those cankers which have a definite line of demarcation about the periphery of the cankerous area outside of which the bark shows no discoloration whatever are usually inactive and seldom carry the pathogene from one season to the next. In some instances, however, the bark surrounding the margin of certain delimited cankers is discolored. Such cankers often become active the following year.

FIGURE 4.—A hold-over fire-blight canker on apple. There is no fissure about the boundary of the canker. The arrow points to the type of location, just outside the discolored area, from which the overwintering bacteria have commonly been isolated

POSITION OF THE BACTERIA IN HOLD-OVER CANKERS

On the basis of work done on the pear, Jones (*17*) states that the blight organisms "live over winter, not in the dead cankered tissue, but in the living bark immediately surrounding the cankered area." Brooks (*5*) concluded from a series of isolations from apple cankers that in the apple, as in the pear, the blight bacteria overwinter, not in the dead tissues of the canker, but in the apparently healthy tissues adjacent to the dead areas. Essentially the same results were obtained by the writer in studies on the position of the bacteria in overwintering apple cankers, although in one instance blight bacteria were isolated from the central portion of a hold-over canker. (Table 2.) Blight bacteria were recovered in a large percentage of the attempts from the tissues adjacent to the discolored margins of the cankers. All efforts to isolate the organism from apparently healthy tissues 2 inches beyond the discolored margin failed. From the work of Jones (*17*), Brooks (*5*), and the writer it seems fairly certain that the blight bacteria do not commonly overwinter in the brown discolored tissues of the canker, but that they are carried from one season to the next in the tissues immediately surrounding the periphery of the discolored margin of the canker. (Fig. 4.)

TABLE 2.—*Results of attempts to isolate Bacillus amylovorus from apple cankers* [a]

Series and dates of platings	Part of canker from which platings were made and results of trials								
	Discolored center			Living tissue 1 to 2 mm. beyond margin			Living tissue 2 inches beyond margin		
	Trials	Results		Trials	Results		Trials	Results	
		Positive [b]	Negative [c]		Positive	Negative		Positive	Negative
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Series 1, Apr. 11, 1926	8	1	7	8	7	1	8	0	8
Series 2, Feb. 28, 1927	25	0	25	24	3	21	25	0	25

[a] Smooth-margined cankers on cut branches of Fameuse variety from Gays Mills.
[b] Positive=*B. amylovorus* isolated.
[c] Negative=*B. amylovorus* not isolated.

Few investigators have made histological studies to determine the mode and place of overwintering of the fire-blight bacteria. Nixon (*21*), after a series of such studies, concluded that *Bacillus amylovorus* overwinters in an encysted condition within the cells. As further information on the mode and place of overwintering seemed desirable, a histological study was made of several smooth-margined cankers collected during the winter. Blocks of tissue, approximately 2 mm. square, from the center of the canker, from the living bark at the periphery of the canker, and from the apparently healthy bark 2 inches from the discolored margin, were fixed in formal acetic alcohol, embedded in paraffin, and sectioned. The sections were stained with rose bengal (Conn, 7) and light green. No bacteria were found in the tissues taken from the central portion of the canker. However, in the tissues taken from the region just beyond the margin of the canker bacteria were found within the sieve tubes of the phloem. In many instances the bacteria were present in such numbers in the cells that the individual rods could be distinguished only with diffi-

FIGURE 5.—A hold-over blighted apple twig; the blight has extended down the twig and affected the two twigs of the current year's growth

culty. However, in no instance has a wall or sac been observed about the bacterial masses other than the cell wall of the sieve tube. The presence of true cysts, therefore, in overwintering blight cankers has not been verified in these experiments. In the apparently healthy tissues 2 inches from the margin of the canker no bacteria were found.

Hold-over Blighted Twigs

Brooks (*5*) has shown that blighted apple twigs play an important rôle as sources of primary inoculum. His observations have, in the main, been confirmed by the writer. In some varieties a comparatively large number of blighted twigs (fig. 5) were found to over-winter the bacteria. In each of the three years that this disease was studied in the field the largest percentage of blighted twigs found overwintering the organism were on trees of the Fameuse variety. (Table 3.)

Table 3.—*The per cent of blighted apple twigs overwintering Bacillus amylovorus in certain apple varieties, Gays Mills, Wis., 1926–1928*

Year and variety	Blighted twigs examined (number)	Overwintering blighted twigs	
		Number	Per cent
1926			
Fameuse	1,114	100	9
McMahon	406	6	1
Wealthy	500	1	0
1927			
Fameuse	1,449	49	3
Wealthy	1,701	2	0
McIntosh	342	1	0
Dudley	614	1	0
1928			
Fameuse	1,581	15	1
Wealthy	500	2	0
Dudley	670	2	0
McIntosh	260	0	0

The size of blighted twigs which overwinter *Bacillus amylovorus* varies considerably. In 1926 the writer measured a number of twigs which had blighted the previous year and which were found to be overwintering the organism. A few of these measured only one-eighth inch in diameter at the point where the discolored tissue merged into the apparently healthy tissue. However, the great majority of overwintering twigs averaged approximately one-fourth inch in diameter. The size of the blighted twigs does not, therefore, limit overwintering.

Relation of the Position of the Hold-over Sources of Primary Inoculum to Subsequent Disease Development

The position of hold-over cankers and overwintering blighted twigs in the trees seems to be of importance in determining the amount of current blossom and twig blight which develops. Observational evidence indicates that in those cases in which the overwintering sources of primary inoculum are found on limbs in the upper portion of the trees a comparatively large amount of both blossom and twig blight usually develops, if conditions are favorable, in the general region

below. On the other hand, in those trees in which the hold-over cankers occur in the crotch or on a limb near the ground line, little primary infection usually develops.

DISSEMINATION OF THE PRIMARY INOCULUM

The exact mode of dissemination of the inoculum which first establishes the fire-blight organism in the current year's growth is, obviously, of great potential importance in relation to control measures. Careful consideration was, therefore, given to the factors concerned in the spread of the primary inoculum.

Relation of Insects

The classic studies of Waite (*30*) clearly demonstrated that bees and other pollinating insects may carry the bacteria from blossom to blossom where they induce blighting. The mode of dissemination of the primary inoculum from hold-over sources to the blossoms and other susceptible parts has not been clearly demonstrated, however, either by observation or experiment. The prevailing idea has been that insects carry the inoculum from cankers where the organism overwinters to the susceptible parts of the current year's growth. Brooks (*5*) has suggested the possibility that some of the initial infection of the fruit spurs and twigs may be brought about by inoculation by aphids. He found them crawling up and down the large branches and also upon the trunks, and he states that "in their wanderings they traveled over exuding cankers and were observed many times to be caught in the bacterial exudate." Observations and experiments conducted by the writer at Gays Mills for three consecutive seasons, however, have failed to confirm the view that insects are important agents of dissemination of the primary inoculum under Wisconsin conditions. In support of this conclusion the following observations and experiments are cited:

(1) Although many hours were spent watching exuding cankers early in the spring in 1926, 1927, and 1928, pollinating or other insects have never been observed by the writer feeding upon or in contact with the exudate coming from the hold-over sources of primary inoculum.

(2) In each of the three years that this disease has been studied in the field there was a dearth of insects in the orchards in the spring and only a relatively few aphids and leaf hoppers were found until later in the season.

(3) Despite the scarcity of insects, numerous blight infections were found in the spring. The blight became apparent early and occurred in all the observed cases beneath hold-over sources of primary inoculum.

In the latter part of May, 1927, a few ants were observed passing over an exuding canker on their way to and from aphids, but extensive observations have failed to indicate that they are of importance in the initial spread of the disease. The rôle of insects in the spread of the primary inoculum appears to be less important, therefore, than has commonly been thought and needs to be reevaluated.

Relation of Meteoric Water

Although Gossard and Walton (*10*) and Brooks (*5*) called attention to the spread of fire blight by meteoric water they did not recognize the importance of this agency as a disseminator of the primary

inoculum. Gossard and Walton (*10, p. 107*) state that "* * * raindrip does not become an agent of dissemination until primary centers of infection have been established, in nearly all cases, by insects." The conditions encountered at Gays Mills, in the seasons of 1926 to 1928, inclusive, have offered a very favorable opportunity to follow the details of dissemination of the primary inoculum under natural conditions. In 1926 and 1927 young blossom clusters and leaf buds were found blighted well in advance of the opening of the blossoms and at a time when there was a striking dearth of insects which might have carried the bacteria from sources of primary inoculum. These diseased blossom and leaf buds were grouped beneath and in the vicinity of exuding cankers or overwintering blighted twigs so that conelike areas of infection resulted, with the sources of primary inoculum at the apices of these areas. The earliest infections of both open-blossom clusters and young shoots have also been observed to occur, almost without exception, below hold-over cankers or twigs in positions favorable for water-borne dissemination of the bacteria from these sources. The further fact was observed that the earliest infections of young shoots became evident at approximately the same time that blossom infections became manifest (figs. 2 and 3), and in many instances blighted blossoms and shoots occurred together in the same infection area.

Additional evidence of the importance of meteoric water in disseminating the primary inoculum was obtained from statistical studies conducted in the field in 1926 to 1928, inclusive. In each of these years the earliest blossom infections were found beneath exuding sources of primary inoculum. A close correlation was found to exist between the abundance of hold-over lesions in the trees and blossom infection. The writer's attention was first directed to this relationship in 1926 during the course of a survey of a group of Transcendents which were growing in the midst of a tract of McMahon apple trees. These Transcendents, of which there were only four, stood within 200 feet of one another, and each tree had approximately the same number of blossom clusters. In two of the trees (Table 4) there was considerable blossom blight, whereas in the other two only an occasional blighted blossom cluster could be found. The diseased clusters in trees 1 and 2 were found only in certain portions of the trees and were grouped in conelike areas of infection. At the apices of these blighted areas hold-over cankers were found.

TABLE 4.—*The relation between the abundance of sources of primary inoculum and the development of blossom blight on Transcendent apple, Gays Mills, Wis., 1926*

Tree No.	Sources of primary inoculum observed		Blossom clusters blighted
	Cankers	Twigs	
	Number	*Number*	*Per cent*
1	3	0	23
2	1	0	15
3	0	0	0
4	0	0	0

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Further evidence of the importance of meteoric water as an agent of dissemination of the primary inoculum was obtained from studies of the relation of the abundance of overwintering sources of primary inoculum to blossom-blight development in Fameuse trees in certain of the control plots at Gays Mills in 1926, 1927, and 1928. In these plots an attempt was made to remove all cankers and blighted twigs while the trees were dormant. In several plots, however, a number of hold-over cankers and overwintering blighted twigs were overlooked during the excision process. Blossom-blight counts made in these plots revealed a close correlation between the abundance of overwintering sources of primary inoculum in the trees and the amount of blossom blight. (Table 5.) In some instances where the hold-over lesions were located on the lower limbs or in the crotches of trees in situations unfavorable for water-borne dissemination of the inoculum this correlation was not so apparent.

TABLE 5.—*The relation of local sources of primary inoculum to the development of blossom blight on Fameuse apple in plots where excision had been practiced,*[a] *Gays Mills, Wis., 1926–1928*

Year and tree No.	Blossom clusters		Hold overs[b]		Year and tree No.	Blossom clusters		Hold overs	
	Examined	Blighted	Cankers	Twigs		Examined	Blighted	Cankers	Twigs
1926	*Number*	*Per cent*	*Number*	*Number*		*Number*	*Per cent*	*Number*	*Number*
1	121	1	0	0	41	151	14	0	1
2	45	0	0	0	42	55	0	0	0
3	52	4	0	0	43	134	33	2	0
4	113	33	1	0	44	129	3	0	0
5	108	44	2	1	45	100	0	0	0
6	100	0	0	0	46	100	0	0	0
7	103	3	0	0	47	100	0	0	0
8	165	48	2	1	48	117	14	1	0
9	129	22	0	1	49	101	1	0	0
10	112	11	0	1	50	63	5	0	0
11	50	0	0	0	51	141	43	3	0
12	50	0	0	0	52	100	0	0	0
13	50	0	0	0	53	107	6	0	1
14	100	0	0	0	54	60	0	0	0
15	50	0	0	0	55	116	1	0	0
16	100	0	0	0	56	70	0	0	0
17	101	1	0	0	57	120	5	0	0
18	77	0	0	0					
19	52	4	0	0	1927				
20	50	0	0	0	1	60	0	0	0
21	86	37	1	0	2	75	0	0	0
22	50	0	0	0	3	51	2	1	0
23	111	10	3	0	4	50	0	0	0
24	70	0	0	0	5	111	1	0	0
25	50	0	0	0	6	85	0	0	0
26	40	0	0	0	7	191	1	0	0
27	75	33	1	1	8	78	4	0	0
28	142	44	3	0	9	127	2	0	2
29	100	0	0	0	10	211	3	0	1
30	104	4	0	0	11	111	1	0	0
31	95	7	0	0	12	278	6	0	0
32	83	19	1	3	13	225	11	0	3
33	96	11	1	0	14	144	6	1	0
34	75	0	0	0	15	141	1	0	0
35	65	0	0	0	16	69	6	0	0
36	127	8	0	0	17	140	0	0	0
37	56	11	1	0	18	80	0	0	0
38	90	55	4	0	19	140	0	0	0
39	51	39	2	0	20	200	0	0	0
40	101	1	0	0	21	85	6	0	1

[a] Blossom-blight counts were made usually the third week after petal fall. In determining the percentage of blossom blight, flower clusters in all parts of the tree were examined so as to obtain representative counts.

[b] Sources of primary inoculum observed. For the most part, the blight infections observed were grouped beneath and in the vicinity of these hold-overs.

TABLE 5.—*The relation of local sources of primary inoculum to the development of blossom blight on Fameuse apple in plots where excision had been practiced, Gays Mills, Wis., 1926–1928*—Continued

Year and tree No.	Blossom clusters		Hold overs		Year and tree No.	Blossom clusters		Hold overs	
	Examined	Blighted	Cankers	Twigs		Examined	Blighted	Cankers	Twigs
	Number	*Per cent*	*Number*	*Number*		*Number*	*Per cent*	*Number*	*Number*
22	105	0	0	0	30	155	6	0	1
23	100	0	0	0	31	75	0	0	0
24	205	44	4	3	32	80	0	0	0
25	135	0	0	0	33	40	2	0	0
26	152	8	0	1	34	150	0	0	0
27	278	1	0	0	35	60	0	0	0
28	112	2	0	0	36	160	0	0	0
29	190	2	0	0	37	40	0	0	0
30	214	2	0	1	38	115	0	0	0
31	76	2	0	0	39	160	0	0	0
32	130	0	0	0	40	60	0	0	0
33	71	1	0	0	41	200	1	0	0
1928					42	155	0	0	0
					43	85	0	0	0
1	110	0	0	0	44	200	0	0	0
2	120	0	0	0	45	100	0	0	0
3	40	0	0	0	46	140	2	0	0
4	150	0	0	0	47	100	8	0	1
5	200	0	0	0	48	100	2	0	[d]2
6	100	0	0	0	49	100	0	0	0
7	200	0	0	0	50	100	0	0	0
8	120	0	0	0	51	100	0	0	0
9	130	0	0	0	52	90	1	0	[c]2
10	130	0	0	0	53	100	0	0	0
11	100	0	0	0	54	100	0	0	0
12	100	0	0	0	55	100	0	0	0
13	110	0	0	0	56	100	17	2	2
14	90	0	0	0	57	100	0	0	0
15	160	11	1	0	58	100	0	0	0
16	140	1	0	0	59	100	0	0	0
17	130	0	0	0	60	110	1	0	0
18	75	1	0	0	61	100	2	0	0
19	175	0	0	0	62	100	0	0	0
20	140	0	0	0	63	100	0	0	0
21	75	0	0	0	64	120	0	0	0
22	90	0	0	0	65	90	9	0	[c]3
23	100	0	0	0	66	45	0	0	0
24	225	0	0	0	67	45	11	0	1
25	60	0	0	0	68	50	0	0	0
26	130	0	0	0	69	85	0	0	0
27	140	0	0	0	70	100	0	0	0
28	60	0	0	0	71	85	0	0	0
29	115	0	0	0					

[c] There were comparatively few blossom clusters underneath the exuding sources of primary inoculum in these trees.

[d] The overwintering blighted twigs in this case were located in the lower portion of the tree. The fact that these sources of primary inoculum were unfavorably situated for water-borne dissemination of the bacteria probably accounts for the small amount of blight found in the trees.

In 1928 large numbers of blight-infection areas were found in late May and early June beneath hold-over lesions. Those trees which contained the largest number of overwintering sources of primary inoculum favorably situated for the water-borne dissemination of the bacteria were commonly found to be the most severely blighted.

From the evidence that has been presented it appears that, under Wisconsin conditions, meteoric water is the most important agency concerned in disseminating the bacteria from hold-over sources of primary inoculum to the current year's growth.

DISSEMINATION OF THE SECONDARY INOCULUM

Following the work of Waite (*30*), Jones (*17*), Stewart (*28*), Burrill (6), and others it became generally accepted that the secondary inoculum is disseminated almost entirely by insects. It was thought that blossom blight was spread chiefly by pollinating insects and that twig blight was disseminated largely by such insects as aphids, leafhoppers, tarnished plant bugs, and others. An important modification of these earlier conceptions was necessitated by the valuable contributions of Gossard and Walton (*10*). From their studies they concluded that from 50 to 90 per cent of the blossom blight which they observed was caused by rain-borne inoculum. In somewhat similar experiments, which appear to have been conducted independently and near the same time, Stevens, Ruth, and Spooner (*27*) showed that blossom clusters and young twigs which were carefully protected from visitation by insects were blighted approximately as much as similar unprotected parts. Access of air and of meteoric water was not precluded. The writers conclude that the disease must have been transmitted by some agency other than insects, and state: "The only tenable hypothesis is that wind was the chief agent of transmission." The present writer's studies furnish additional evidence that meteoric water is an important agent in the dissemination of the secondary inoculum for fire blight and that the rôle of insects in the spread of the disease is less important than was earlier believed. In support of this view the following observations and experiments are pertinent.

(1) In each of the three years that this disease was studied under natural conditions, trees varied greatly in the severity of blossom blight, and blighted clusters were often found grouped in conelike areas of infection. In some instances practically every blossom cluster in one part of a tree was infected, whereas in other portions of the same tree only scattered clusters were diseased. No hold-over sources of primary inoculum could be detected in certain trees showing such conelike areas of infection. Instead of hold-over lesions, early blighted blossom clusters were found to be the source of inoculum. If all blossom infections are the result of inoculations by insect carriers of fire blight a more uniform distribution of blight infections in the trees should be expected with regard both to the trees infected and the distribution of blighted blossoms on individual trees.

Many other observations pointed to a more localized spread of blight than would be expected if pollinating insects were the chief disseminators of the secondary inoculum. In 1927, for example, a considerable local development of blossom blight was found in one corner of a McMahon tract, the remainder of which showed a very sparse development of blossom blight. The trees which were heavily infected were grouped about a badly blighted Transcendent in which were found hold-over lesions. No hold-overs were observed in the badly blighted McMahon trees and only rarely occasional cases of overwintering of the organism were found on the McMahons in this block. The amount of blossom blight diminished rapidly as the distance from the Transcendent increased.

A similar example on a larger scale was observed in the same year. A large block of Fameuse which contained numerous hold-over lesions and was heavily infected with blossom blight adjoined a block of Dudley in which only occasional hold-over lesions were found. The

blooming periods of the two varieties were approximately identical. (Fig. 2.) In the Dudley trees immediately adjacent to the Fameuse block abundant blossom blight developed. The amount of blight, however, diminished rapidly as the distance from the Fameuse block increased, most of the disease being confined to five or six rows nearest the Fameuse. The more remote parts of the Dudley block, including some 10 acres, remained practically free from blossom blight.

FIGURE 6.—Bacterial exudate coming from the pedicel of a pear flower inoculated by atomizing a pure culture of *Bacillus amylovorus* into the receptacle cup. Photograph taken three days after inoculation

(2) Attention was further directed to the important rôle played by meteoric water in the secondary spread of fire blight in experiments which were conducted in 1928 at Gays Mills. Two apple trees, a Fameuse and a Wealthy, containing numerous blossom buds in the early closed-cluster stage of development, were sprayed with Derrisol (1-800) to kill any aphids which might have been present. These trees were then inclosed in cheesecloth insect-proof cages with the aim of excluding all pollinating and other insects. Several early blooming flower clusters in the top of each of the trees were inoculated on May 10 with a pure suspension of *Bacillus amylovorus* in sterile distilled water by carefully inserting the inoculum into the receptacle cups. Bacterial exudate was found on May 15 coming from the pedicels of the inoculated flowers. Three-tenths inch of rain fell the following day (May 16), and on May 18 there was a precipitation of nine-tenths inch. On May 23, five days after the last of these two rains, a number of flower clusters were found beneath the inoculated ones showing fire-light symptoms. Conelike areas of infection developed with the inoculated flower clusters at the apices of these diseased areas. (Fig. 7.) Examinations failed to reveal the presence of any

FIGURE 7.—A blighted Fameuse apple tree. Before the blossoms opened this tree was inclosed in a cheesecloth cage to keep out pollinating insects, and two blossoms clusters at X were inoculated. Abundant secondary infection of blossom clusters developed in a cone-shaped area. Each infected blossom has a white tag attached

insects. Meteoric water, therefore, appears to have been the only agent which could possibly have disseminated the inoculum.

(3) In 1926 at Gays Mills aphids were scarce in the spring but numerous in midsummer. In 1927 both leaf hoppers and aphids were relatively numerous from the middle of June throughout the growing season. In spite of their relative abundance, however, twig blight was not at all serious either in 1926 or 1927, except in a relatively small number of trees scattered here and there throughout the orchard. In virtually all of the trees which were infected either blighted blossom clusters or overwintering sources of primary inoculum were discernible in some part of the trees. This relationship was very apparent in certain trees in some of the plots at Gays Mills in which an attempt was made to remove all overwintering sources of inoculum in the fall of 1925. The following spring several trees in these plots were found to contain an abundance of both infected blossoms and shoots. The disease development observed in these trees was found upon examination to be traceable to overwintering cankers which had been overlooked during the excision process the preceding fall. The adjoining trees, while heavily infested with leaf hoppers, contained only a scattered infection here and there. If the widespread dissemination of twig blight is to be attributed largely to sucking insects, theoretically we should expect a cross transfer of the inoculum from heavily infected trees to adjacent ones. This is not in accord with the writer's observations, which extend over a 3-year period. It would seem, therefore, that a local source of inoculum either in the form of hold-over cankers, overwintering blighted twigs, or diseased flower clusters or shoots must be present in the trees before twig blight becomes serious. (Table 6.)

TABLE 6.—*The relation of the number of blighted blossom clusters in Fameuse apple trees to twig-blight development, Gays Mills, Wis., 1927*

Tree No.	Blighted blossoms	Blighted twigs	Tree No.	Blighted blossoms	Blighted twigs
	Number	*Number*		*Number*	*Number*
1	9	11	22	15	39
2	13	6	23	1	2
3	0	1	24	0	1
4	3	3	25	0	0
5	1	2	26	0	2
6	0	0	27	0	0
7	0	1	28	0	0
8	2	0	29	0	0
9	0	0	30	0	0
10	0	0	31	0	0
11	3	1	32	14	10
12	9	5	33	2	4
13	2	9	34	120	29
14	12	11	35	4	24
15	0	0	36	0	0
16	0	0	37	0	0
17	0	0	38	0	1
18	7	8	39	0	1
19	2	6	40	0	1
20	14	56	41	3	0
21	51	6	42	0	1

While the work of Waite (*30*), Gossard and Walton (*10*), and others has shown that pollinating insects may spread fire blight, there are practically no data available which would clearly indicate the

extent to which they are concerned in the dissemination of the disease. Does a bee disseminate the disease extensively after visiting a diseased flower, or does it cease to be a carrier of blight after visiting a comparatively few flowers? This is a very important point in relation to the epidemiology of fire blight and deserves critical consideration. In 1928 the writer made a preliminary attempt to gain evidence on this problem, but due to the limited amount of material available only a beginning was made toward its solution. In experiments conducted in the greenhouse the proboscis of a bee was dissected out and tied to the end of a small stick of wood. The tip of the distal end was then dipped in melted paraffin in such a manner as to seal the opening to its interior. The proboscis and its carrier were sterilized in 95 per cent alcohol and washed through several changes of sterile water. The distal portion of the proboscis was then dipped into the nectarial fluid of a pear flower which had been inoculated with *Bacillus amylovorus* three days earlier. The proboscis was next dipped consecutively into the nectar in the receptacle cups of 15 healthy pear flowers. Every one of the flowers thus inoculated blighted in a very characteristic manner. This would seem to suggest that pollinating insects may still be potential agents of dissemination even after having visited 15 flowers following contamination.

THE MODE OF ENTRY OF BACILLUS AMYLOVORUS

INTO YOUNG SHOOTS AND UNOPENED FLOWERS

It has long been accepted that access of *Bacillus amylovorus* to the host tissues is accomplished in two ways: (1) Through the open blossoms, presumably through the uninjured nectaries in the receptable cup (Waite (*30*, *31*)) and (2) through wounds of many kinds (Jones (*17*), Stewart (*28*), Burrill (*6*), Heald (*13*), and others). On the basis of observations, Heald (*13*) expressed the opinion that the bacteria may gain entrance to the leaves also through water pores and stomata but stated that it remained for further investigation definitely to subtantiate this view. He states (*14*, *p. 315*):

> The writer studied fire blight in Washington in the summer of 1915 and found leaf invasions common in pear, apple, and quince, and later produced artificial infections through the leaf margins by the use of pure broth or bouillon cultures. Leaf infections occurred through marginal breaks, insect punctures or through perfectly sound leaves.

Brooks (*5*) reported negative results from numerous inoculation experiments with unwounded apple leaves in the field and greenhouse. Most of this work was done, however, after the twigs had made considerable growth.

The occurrence at Gays Mills of blighted cluster buds and infected young shoots early in the season in the absence of insects and in association with hold-over sources of primary inoculum suggested to the present writer the hypothesis that the bacteria are able to infect young shoots and unopened blossoms without the intervention of wounds. Attention was, therefore, directed to the manner in which the bacteria gain access to the tissues of the host. Working with potted apple and pear trees grown in the greenhouse under conditions designed to prevent injury from insects and other agencies, the writer

induced infection of young shoots and unopened blossom buds of apple and pear at will by spraying them with a pure suspension of *Bacillus amylovorus* in sterile distilled water and placing them for varying periods in a moist chamber [5] at suitable temperatures. After inoculation the plants were incubated in a well-illuminated chamber [6] which was maintained at a temperature of 28° C. and 70 per cent relative humidity. Two types of disease development commonly followed. In those varieties which have a thick mat of hairs on the dorsal surface of the young leaves (as Wealthy and Fameuse) the first symptoms of the disease commonly appeared along the margin of the young leaves from four to six days after inoculation. From the margin the disease spread down the midrib if the lesions were terminal,

FIGURE 8.—Representative twigs grown under conditions designed to prevent injury, inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water, and demonstrating the entry of the fire-blight bacteria through stomata. Twigs on the tree at the left (A) are controls

or the veinlets if lateral, resulting in lesions which were more or less triangular in outline. The petioles of the infected leaves were then invaded, followed by infection of the main axis of the shoot. In a comparatively short time a decided wilting and typical blighting of the young shoots developed. (Fig. 8.) Bacterial exudate was found on the invaded areas in many instances. *B. amylovorus* was successfully recovered from the invaded areas in all of the numerous isolations attempted. In those varieties which have comparatively few hairs on the undersurface of the young leaves (as Transcendent or Whitney)

[5] Through the kindness of Dr. G. W. Keitt, an infection chamber (Keitt and Jones (*18*)) was made available for these studies. Slight alterations were made in the method of maintaining the humidity. The desired temperature and humidity in the chamber were maintained in part by 250-watt luminous radiator unit covered with moist absorbent cotton. Moistened pieces of cheesecloth suspended from troughs of water around the walls of the chamber replaced the cloth inner chamber used by Keitt and Jones and aided in keeping the desired temperature and humidity. Hot or cold water from supplies kept at suitable constant temperatures were mixed through metal valves to give the desired temperature to the water in the troughs.

[6] The writer is indebted to Dr. J. G. Dickson for his kindness in permitting the use of one of his temperature and humidity chambers for these studies.

a leaf spotting (fig. 9) often occurred, particularly, if a premoist treatment had been given the young shoots before inoculation. These lesions often coalesced and the disease invaded the midrib, petiole, and main axis of the shoot in a manner comparable to that outlined above. These two types of development following inoculation of young shoots were associated, in part at least, with the wettability of the dorsal surfaces of the young leaves. The older leaves seemed to be more or less resistant to the invasion of the bacteria, for only a very few became infected until the main axis of the shoot was invaded. In those that were infected the blight lesions were localized at the margins and failed to spread to any considerable extent. The factors which are responsible for the apparent resistance of the older leaves have not been fully analyzed.

FIGURE 9.—Leaf from a young shoot of Whitney (crab) apple inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water; a leaf-spot symptom was commonly observed on this variety in greenhouse experiments during the earlier stages of disease development

Blossom buds inoculated and treated in the same manner as the young shoots showed symptoms of fire blight within three to six days. The tips of the exposed sepals and the apices of leaves surrounding the blossom buds turned brown (fig. 10), and under humid conditions a bacterial exudate was found coming from the discolored parts. In a number of instances isolations were made from the invaded areas with uniformly successful results. Four to six days after the appearance of the first symptoms of the disease, the tissues of many of the infected buds were all brown and dead, and evidence of the disease, in many instances, was found also in the 1-year-old wood. As is shown in Table 7, in those cases where the buds received a preliminary moist treatment before inoculation there was a larger percentage of infections than when no premoist treatment was given. However, in view of the positive results obtained without the administration of a preliminary moist treatment it seems that such treatments are not absolutely essential for infection.

FIGURE 10.—Cluster of infected unopened apple blossoms. These buds were inoculated by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water. The apical parts of the sepals (*a*) and of one leaf (*b*) are diseased

TABLE 7.—*Results of inoculation experiments with Bacillus amylovorus on unopened apple-blossom buds and young shoots grown under conditions designed to prevent injury from insects and other agencies, Madison, Wis., 1928*

Parts inoculated and number of experiment	Period in moist chamber		Results				
			Inoculations			Controls	
	Before inoculation	After inoculation	Healthy	Diseased	Organism reisolated	Healthy	Diseased
	Hours	*Hours*	*Number*	*Number*		*Number*	*Number*
Twigs:[a]		48	1	2	[b]+	4	0
1		96	1	1	+	5	0
		168	0	7	+	2	0
2		72	3	1	+	3	0
		96	3	1	+	4	0
3	48	24	2	7	+	6	0
4	46	24	0	4	+	4	6
5	40	26	2	9	+	2	0
6	26	25	1	4	+	6	0
7		24	4	9	+	8	0
	22	25	4	6	+	11	0
8	24	24	0	9	+	3	0
9	19	24	0	2	+	1	0
Blossom buds:[c]							
1		24	0	1	+	2	0
	41	24	0	2		3	0
2		22	0	3	+		
	23	22	0	7		5	0
3		22	5	1	+		
	19	22	1	4		5	0
4		28	0	1	+	1	0
	16	28	0	3		3	0
5		23	0	2	+	2	0
6	20	24	1	2	+	3	0
7	24	27	0	6		1	0
8	6	25	0	2			

[a] Young succulent shoots, 2 to 7 inches long.
[b] + = *B. amylovorus* reisolated.
[c] Apple blossom buds in the green tip and early closed cluster stage of development.

In other experiments relating to the possibility of infection of the host tissues by *Bacillus amylovorus* without the intervention of wounds, the stamens and styles of open apple flowers, grown in the greenhouse under conditions designed to prevent injury by insects or other agencies, were removed with a pair of scissors and the receptacle cups filled with cocoa butter in such manner as to cover the nectaries and the wounds made incident to this excision. In all, 73 flowers were treated in this manner during the course of the experiment. After the cocoa butter had hardened, pure suspensions of *B. amylovorus* in sterile distilled water were sprayed upon the sepals and outer sides of the receptacle cups. The plants were then placed in the inoculation chamber for 24 hours, after which, in most instances, they were transferred to a chamber which was maintained at 28° C. and 70 per cent relative humidity. There they were left during the remainder of the experiment. In a number of instances where flowers and young fruits were inoculated in this manner, brownish, slightly depressed lesions (fig. 11) developed on the sides of the fruits after a period ranging from 5 to 10 days following inoculation. *B. amylovorus* was success-

FIGURE 11.—Fire-blight lesions on young apple fruits inoculated 10 days after petal fall by atomizing with a pure suspension of *Bacillus amylovorus* in sterile distilled water

fully recovered from the invaded areas in all of the reisolations attempted from these lesions. The margins of the diseased areas, in many instances, were extended until the entire fruit was invaded. The results of these studies are given in Table 8.

TABLE 8.—*Results of inoculation experiments with Bacillus amylovorus on apple flowers from which the stamens and styles were removed and the receptacle cups filled with cocoa butter before inoculation, Madison, Wis., 1928*

Experiment No.	Period flowers were in moist chamber		Total flowers inoculated	Results of inoculation	
	Before inoculation	After inoculation		Plants remaining healthy	Diseased plants
	Hours	*Hours*	*Number*	*Number*	*Number*
1		24	67	35	32
2		23	2	0	2
	15	48	2	1	1
	24	23	2	0	2

Field studies of the infection of unopened blossoms by *Bacillus amylovorus* were also made in 1927 and 1928. A number of blossom buds in the early and middle closed-cluster stages of development were sprayed with nicotine sulphate (1–800) to kill any aphids which might be present. Later in the day, and, in some instances, on the following day, these unopened blossoms were sprayed with a pure suspension of *B. amylovorus* in sterile distilled water. These inoculated unopened blossoms were then caged in celluloid chambers in such a manner as to prevent the parts from coming in contact with any surface which might injure them. The results of these experiments are given in Table 9.

TABLE 9.—*Results of inoculations made by spraying Bacillus amylovorus [a] on unopened blossoms,[b] Gays Mills, Wis., 1927–28*

Date of inoculation	Variety inoculated	Inoculum applied with—	Blossom buds [b] inoculated	Number blighted	Per cent blighted
1927			*Number*		
May 4	Fameuse	Camel's-hair brush	14	3	21
May 13	Transcendent	Atomizer	130	31	24
May 16	McIntosh	do	37	0	0
May 20	Wealthy	do	23	0	0
May 25	Fameuse	do	154	4	3
1928					
Apr. 4	Transcendent	do	295	102	34
Apr. 6	do	do	82	42	51
Apr. 11	McMahon	do	60	0	0
Apr. 15	McIntosh	do	60	0	0
Apr. 23	Wealthy	do	17	0	0
Apr. 24	McIntosh	do	42	0	0
Apr. 26	do	do	50	0	0
Apr. 28	do	do	46	1	2
May 1	Wealthy	do	17	11	64
May 3	McIntosh	do	20	4	20
May 4	Wealthy	do	13	0	0
Do	McIntosh	do	47	4	9
May 5	Wealthy	do	10	2	20
May 6	McIntosh	do	12	0	0

[a] The inoculum consisted of pure suspensions of *Bacillus amylovorus* from broth cultures in sterile distilled water.

[b] The blossom buds inoculated ranged from those in the green tip to those in the open-cluster stage of development.

In field experiments carried on in 1928 unopened blossoms and young shoots, after a preliminary spraying with Derrisol (1–800) to kill any aphids which might be present, were given premoist treatments in specially constructed metal chambers mounted on tripods and lined with moist absorbent cotton. (Fig. 12.) A pure suspension of *Bacillus amylovorus* in sterile distilled water was then sprayed upon the young shoots and unopened blossoms and the inoculated parts placed in moist chambers in such a manner as to prevent injuries to the tissues. The results of these studies are given in Table 10.

TABLE 10.—*Results of spraying Bacillus amylovorus* [a] *on unopened blossom buds and young shoots* [b] *which were subjected to moist treatments before and after inoculations, Gays Mills, Wis., 1928*

Parts inoculated and dates of inoculation	Variety inoculated	Period in moist chamber		Total inoculated	Number blighted	Per cent blighted
		Before inoculation	After inoculation			
Blossom buds: [c]		*Hours*	*Hours*	*Number*		
Apr. 23	Wealthy		48	6	0	0
Apr. 24	Dudley	18	24	20	0	0
Do	McIntosh	18	24	20	0	0
Apr. 26	do	24	24	15	0	0
Do	Dudley	24	24	12	0	0
Do	McMahon	24	24	13	0	0
Apr. 28	do	48	48	11	0	0
Do	McIntosh	24	24	36	0	0
May 1	do	46	24	13	4	30
Do	Wealthy	46	24	21	7	33
May 3	McIntosh	24	24	8	7	88
Do	do	48	48	17	2	12
Do	Wealthy	24	24	22	4	18
May 5	do	24	24	21	5	24
Do	McIntosh	24	24	8	7	88
May 6	do	24	24	10	1	10
May 7	do	24	24	4	2	50
Do	Wealthy	24	24	21	4	19
May 9	do	24	24	14	8	57
Twigs: [d]						
May 8	do		24	10	6	60
May 10	do	48	24	5	3	60
May 11	do		24	15	7	47
May 14	Wealthy		24	10	0	0
Do	Transcendent		24	9	0	0
May 16	do			37	1	3
Do	Wealthy		24	5	1	20
Do	do			11	3	27
May 17	do			24	4	17
Do	Transcendent			18	0	0
May 18	do		24	7	0	0
Do	do	24	24	20	1	5
Do	Wealthy	24	24	20	0	0
May 20	do	24	24	7	1	14
Do	McMahon		24	9	0	0
May 21	do	8	24	8	0	0
May 24	Wealthy	24	24	4	0	0

[a] The inoculum consisted of pure suspensions of *B. amylovorus* from broth cultures in sterile distilled water.

[b] The parts inoculated were grown under conditions designed to minimize the chance of injury by insects or other agencies as described above.

[c] The blossom buds inoculated ranged from those in the green-tip to those in the open-cluster stage of development.

[d] The young shoots inoculated were from 2 to 7 inches long.

As is shown in Tables 9 and 10, the percentage of positive results was much more variable in the field experiments than in the greenhouse tests where conditions could be controlled more satisfactorily. Positive results were sufficiently numerous, however, to give strong indica-

tion that, under field conditions, *Bacillus amylovorus* can gain entrance to the host tissues through natural avenues of entry other than those of the inner (ventral) surface of the blossoms.

In an effort to ascertain more definitely the mode of entry of *Bacillus amylovorus* under the conditions of these tests, a detailed histological study was made of the tissues of young leaves and unopened blossoms which had been inoculated in the greenhouse by spraying with a pure suspension of *B. amylovorus* in sterile distilled water. Cases of stomatal penetration were found in tissues taken from the dorsal side of inoculated young apple leaves (fig. 13, A, and fig. 14, B) and from the sepals of inoculated unopened apple flowers. (Fig. 13, D.) The characteristic marginal invasion of young leaves suggests that the bacteria may gain access to the host tissues also through water pores, although the writer has thus far failed to find the bacteria entering these natural openings. The data which have been reported justify the conclusions: (1) That *B. amylovorus* can, under suitable conditions, infect young shoots and unopened blossoms of apple without the intervention of wounds, and (2) that, under some conditions, *B. amylovorus* can cause infection following penetration through stomata.

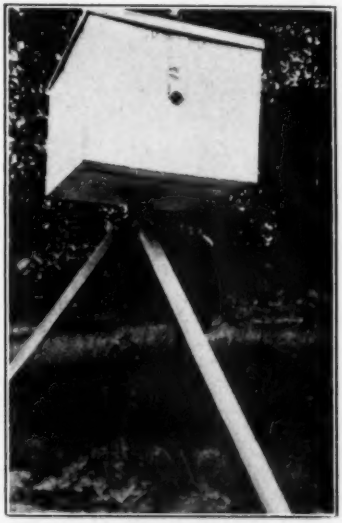

FIGURE 12.—Inoculation chamber used in the field experiments; the metal box was lined with moist absorbent cotton.

INTO OPEN FLOWERS

Following the work of Waite (*30*, *31*), it has been generally accepted that *Bacillus amylovorus* gains access to the tissues of the open blossoms through the nectaries in the receptacle cup. So far as the writer is aware, however, no histological study has ever demonstrated their actual entry into the tissues through the nectaries. It seemed desirable, in view of the lack of definite histological evidence, to trace the avenue of entry into the tissues of open blossoms. In these studies pure broth cultures of *B. amylovorus* were sprayed into the receptacle cups of a number of open apple and pear flowers. Fixations were made in formal acetic alcohol at short intervals after inoculation. The flowers were sectioned, after embedding in paraffin, and stained with rose bengal and light green. In both apple and pear flowers, which had been inoculated 30 hours before fixations were made, the bacteria were found gaining access to the host tissues through openings in the receptacle cups which appear to be stomata. (Fig. 13, B and C; fig. 14, A.) In none of the flowers sectioned by the writer thus far have the bacteria been found gaining access to the tissues of open flowers except by entering stomata. The writer has been unable to find evidence in the literature (see Eames and McDaniels (*9*, *p. 78*)) or in his own preparations that the floral nectaries

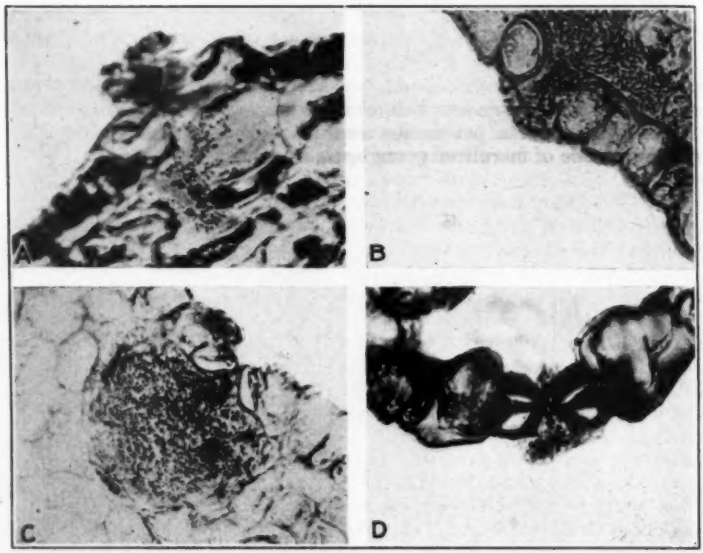

FIGURE 13.—Photomicrographs of host tissues invaded through stomata by *Bacillus amylovorus*. The inoculations were made by atomizing a pure suspension of the blight bacteria in sterile water. The bacteria are shown in the stomatal openings and in the substomatal chambers. A, Cross section of an apple leaf; B, longitudinal section of the receptacle cup of an apple flower; C, cross section of the receptacle cup of a pear flower; D, cross section of a sepal of an unopened apple flower

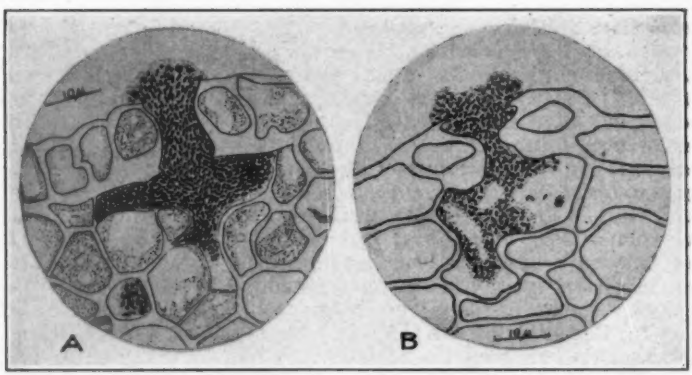

FIGURE 14.—Camera lucida drawings of host tissues invaded through stomata by *Bacillus amylovorus*. A, From the section shown in Figure 13, B; B, from the section shown in Figure 13, A

of apple or pear offer an open avenue for the entry of bacteria, as no intercellular spaces which pass from the surface through this tightly compacted layer have been demonstrated.

THE MIGRATION AND PARASITISM OF BACILLUS AMYLOVORUS

A number of valuable studies have been made of the relation of *Bacillus amylovorus* to its host. As most of these studies are readily accessible through the works of Stewart (*28*), Bachmann (*4*), Nixon (*21*), and Haber (*11*), an extended review of this literature appears to be unwarranted. However, notwithstanding the many excellent contributions which have been made to the knowledge of the intimate relationships existing between the causal organism and its hosts, many problems still remain unsolved. It is not at all clear just how the bacteria cause the death of the host cells. Do the bacteria secrete toxins or is the death of the cells due to the plasmolysis of the protoplast following the extraction of water from the cell? Still less is known as to how the bacteria gain access to the cells and how they migrate from one cell to another. The conditions surrounding the formation and extrusion of the well-known bacterial exudate have never been adequately described. Furthermore, considerable question still attaches to certain details of the mode of overwintering of the bacteria. While a comprehensive study of these problems could not be included in the present work, some attention was given them in the hope of contributing to their advancement, rather than their full solution.

STUDIES RELATING TO THE MANNER IN WHICH THE HOST CELLS ARE KILLED

A study of the literature reveals two views as to the manner in which the host cells are killed. Bachmann (*4*), Nixon (*21*), and others think that the cells are probably killed by the extraction of water from them and its passage into the intercellular spaces, with consequent plasmolysis. Stewart (*28*), however, is of the opinion that toxic products may be secreted by the pathogene which upon diffusion kill the cells.

The results of histological studies conducted by the writer accord with those of Bachmann (*4*) and Nixon (*21*) in showing that in early stages of migration the bacteria in the intercellular spaces were often found completely surrounding host cells without any noticeable ill effects other than a slight shrinking of the cell contents. Additional support to the view that plasmolysis following loss of water is an important factor in the death of the host cells was gained from experiments set up to determine whether the bacteria could thrive in solutions which had sufficient osmotic pressure to kill the host cells. A sucrose solution having an osmotic pressure of 20.73 atmospheres was made up and divided into two portions. Free-hand razor sections from a young apple leaf were placed in one of the portions and bacteria from a 3-day-old potato dextrose culture of *Bacillus amylovorus* in the other. At intervals microscopic examinations were made of the cells in the sugar solution. After remaining in the solution for one hour almost every cell in the section was plasmolyzed. The cells in control leaf sections in sterile distilled water, on the other hand, were turgid. Dilution plates were poured from the bacterial suspension in the sugar solution, and a large number of bacterial

colonies appeared on the agar plates after a suitable incubation period. There were live bacteria in the sugar solution even after a 24-hour exposure. This evidence indicates that the fire-blight bacteria can exist in the intercellular spaces in solutions having osmotic pressures sufficient to plasmolyze the living host cells.

In experiments conducted to determine whether toxins are produced by *Bacillus amylovorus*, Arthur (*3*) failed to detect the presence of toxic substances in filtered culture solutions in which the fire-blight bacteria had been growing. As further experimental evidence of the production or nonproduction of toxins by *Bacillus amylovorus* seemed desirable, the writer attempted to isolate toxic substances from (1) diseased host tissues and from (2) culture solutions in which the bacteria had been growing. Twigs affected with fire blight were ground up and the juice was extracted from the crushed tissue by squeezing through a cheesecloth. The juice was then filtered through a Berkefeld filter, and cross sections from a surface-sterilized apple leaf cut with a flamed razor were placed aseptically in (1) the filtrate and (2) sterile distilled water. At approximately 12-hour intervals, sections in the filtrate and in the controls were removed and examined under the microscope. No appreciable difference in the material from the filtrate and from sterile distilled water was noticed even after a 48-hour exposure. No evidence of toxic action was observed.

The possibilities of the production of exo-toxins which are precipitable by alcohol was next considered. In these studies 95 per cent alcohol was added to an 8-day-old nutrient broth culture and the resulting precipitate collected on filter paper, dried under aseptic conditions, and redissolved in sterile distilled water. This solution was then studied for toxic action as outlined above. No toxic action was detected. These experiments were repeated with the same results. This study suggests, therefore, that toxins of the sort which can be isolated by the methods used are not produced by *Bacillus amylovorus*.

STUDIES OF THE MIGRATION OF BACILLUS AMYLOVORUS IN THE HOST TISSUES

There has been some difference of opinion concerning the mode of migration of *Bacillus amylovorus* through the host tissues. Stewart (*28*), Bachmann (*4*), and certain other investigators seem to think that the bacteria migrate in the intercellular spaces in a free-swimming condition. Nixon (*21*) and Haber (*11*), however, take the view that they migrate through the tissues as zoogloea. Nixon (*21*) states that these zoogloea consist of jellylike matrices with blunt, more or less rounded ends, densely packed with bacteria. He further states that they migrate by a growth movement which parallels in many respects the movement of pseudoplasmodia of the Myxobacteriaceae.

In order to gain more evidence regarding the mode of migration of *Bacillus amylovorus* in the host tissues histological studies were made. A number of young shoots on potted Wealthy apple trees were inoculated in the greenhouse by puncturing near the tips with a fine needle dipped in a broth culture of the pathogene. The inoculated trees were placed in a moist chamber in the greenhouse at 25° C., and fixations were made in Flemming's strong fixing fluid at intervals of 1, 3, 5, 10, and 24 hours, respectively, after inoculation. Histological studies were then made of sections from this material, using Flemming's triple stain. In the twigs fixed 1 and 3 hours, respectively,

after inoculation the bacteria were found in the intercellular spaces, but no evidence was obtained to indicate that they were embedded in a jellylike substance. Their distribution and appearance suggested that they were in a free-swimming condition when the fixations were made. In the twigs fixed 10 and 24 hours, respectively, after inoculation, however, the bacteria were found in the intercellular spaces of the cortex embedded in a substance which stains differently than do the bacteria. Nixon (*21*) interprets these masses as zoogloea. Such interpretation would seem to depend on one's conception of zoogloea. This conception, as usually defined by bacteriologists, provides that the slimy matrix in which the bacteria are embedded is secreted by the bacterial cells. The evidence thus far adduced has not shown conclusively that, in the case of the fire-blight organisms in the host tissues, the matrix under discussion is of bacterial origin. Arthur (*3*), in his studies on the biology of pear blight, illustrates zoogloeal formations. These so-called zoogloea were probably due to the presence of other organisms in his cultures, as Snyder (*25*), Stewart (*28*), and others have since failed to demonstrate the production of zoogloea by *Bacillus amylovorus* in pure culture. Until further evidence to the contrary is adduced, the possibility seems to remain that the fire-blight bacteria, upon being introduced through wounds into susceptible host tissues, first migrate in a free-swimming condition and later consolidate as they increase in numbers. Further study of the detailed mode of migration of the organisms and of the origin of the matrix in which they lie seems desirable.

FIGURE 15.—Photomicrograph of a group of infected phloem cells in an active blight canker; the bacteria are shown within the cells and appear to have migrated from one cell to another through an aperture in the cell wall

Histological evidence suggests the production of enzymes and their activity in the development of the pathological condition. In stained sections of young apple shoots and flowers inoculated with *Bacillus amylovorus* the intercellular spaces invaded by the bacteria were, as a rule, larger than those in the corresponding normal tissues. In many instances bacteria were observed in invaded tissue completely surrounding the cells, which would seem to indicate that the substances which cement the cells together had been dissolved. Such evidence suggests that an enzyme of the nature of pectinase is produced either by *B. amylovorus* or by the host cells under the stimulus of bacterial invasion. However, the fact that no decided softening of the tissues ensues would suggest that any such enzymatic action occurs on a very small scale.

In histological studies of blight cankers the bacteria were found migrating from one cell to another through openings in the cell walls (fig. 15) which appear to have been formed by a dissolution of the wall substances. The portion of the wall which appears to

have been dissolved is, in some instances, quite limited and localized. This fact suggests a mass action of the bacteria. In certain invaded areas, however, as in the phloem, comparatively large cavities were found in which the bacteria were present in great numbers. Histological evidence suggests that a solvent action on cell walls played a part in the formation of these cavities. It seems probable that either the parasite or the host under its stimulus secretes very small amounts of an enzyme of the nature of cellulase.

Pressures which may develop in the intercellular spaces incident to the growth of the bacteria and to the physiological processes of host and parasite may possibly play some part in the development of the tissue changes which have just been discussed. However, the sharp localization of many of the changes observed and certain details of the histological picture, as orifices in cell walls bounded by well-rounded edges (fig. 15) and a scarcity of evidence of the crushing of cells, make it difficult to explain the observed phenomena wholly on the basis of pressures developed outside of the host cells.

Experiments were made with pure cultures of *B. amylovorus* with the aim of isolating any exo-enzymes which are precipitable by alcohol. In these experiments 95 per cent alcohol was added to an 8-day-old nutrient broth culture of *B. amylovorus* and the resulting precipitate collected on filter papers, dried under aseptic conditions, and redissolved in sterile distilled water. Pieces of carrots cut aseptically from carrot roots were dropped into the redissolved precipitate and at the same time other pieces of carrots were placed in tubes of sterile distilled water as controls. At approximately 24-hour intervals, microscopic examinations were made of thin razor sections from the carrot tissues in the redissolved precipitate and from those in the controls. After 24 hours had elapsed no significant difference could be detected between the sections in the redissolved precipitate and the controls in sterile distilled water. Differences were discernible, however, after 72 hours of exposure. The cell walls of the carrot tissue exposed to the action of the redissolved precipitate were more swollen and the intercellular spaces appeared to be larger than those in the carrots immersed in sterile water. This would seem to indicate that the pectic layers were being slowly dissolved by enzymes which had been secreted by the bacteria. The fact that this action is so slow, however, suggests that only small amounts of exo-enzymes precipitable by alcohol are secreted by *B. amylovorus*. There is no evidence of a rapid dissolving action as is the case with the soft-rot organism, *B. carotovorus*.

An attempt was also made to ascertain whether cellulase is produced by pure cultures of *B. amylovorus*. In these studies pure cultures of the pathogene were grown on cellulose agar for a period of 10 days. There was no indication that the cellulose was being fermented by the bacteria. Furthermore, no action was noticeable on pieces of filter paper even after a 30-day exposure to the action of a nutrient broth culture of *B. amylovorus*. The results of these studies suggest that cellulase is not produced by *B. amylovorus* in significant amounts in cultures of the kind used in these studies. However, there still remains the possibility that the pathogene produces an enzyme of the nature of cellulase in association with the host. It may also be that cellulase is not secreted by *B. amylovorus*, but is produced by the host cells under the stimulus

of bacterial invasion. Unless the production of small amounts of some cell-wall dissolving substance in the invaded tissues either by the host or the parasite is postulated, the evidence presented by histological studies of the diseased tissues is without adequate interpretation.

VARIETAL RESISTANCE OF APPLES TO FIRE BLIGHT

No variety of apple grown in Wisconsin has been found to be totally immune to fire blight. However, it has long been observed that some varieties suffer much less than others from this disease. This was shown rather strikingly in studies of the relative abundance of blossom infections in a number of different apple varieties growing in the orchards at Gays Mills. Based on counts made in 1926, 1927, and 1928, Transcendent, McMahon, Fameuse, and Wealthy are more subject to blossom-blight attacks, under Wisconsin conditions, than McIntosh, Northwestern Greening, or Dudley. (Table 11.) It is a rather significant fact that those varieties which are most subject to blossom infection have, as a rule, the most overwintering sources of primary inoculum. (See Table 1, p. 584.)

TABLE 11.—*The per cent of blossom blight on some apple varieties, Gays Mills, Wis., 1926–1928*

Year and variety	Blossom clusters [a]—		
	Examined (number)	Blighted	
		Number	Per cent
1926			
Transcendent	925	228	24
Fameuse	24,805	5,055	21
Wealthy	46,182	3,225	7
Northwestern Greening	2,112	64	3
McIntosh	1,208	28	2
1927			
McMahon	19,696	3,949	20
Fameuse	19,205	1,816	9
Wealthy	19,416	1,364	7
Dudley	12,107	363	3
McIntosh	19,620	561	3
Northwestern Greening	13,013	333	3
1928			
Fameuse	16,000	420	3
Wealthy	16,000	381	2
McIntosh	16,000	382	2
Dudley	16,000	204	1
Northwestern Greening	16,000	94	1

[a] The blossom clusters were examined the third and fourth weeks after petal fall. The method used for determining the percentage of blossom blight consisted in examining from 50 to 100 blossom clusters representatively distributed in all parts of the tree in each of approximately 100 representative trees of each variety.

INFLUENCE OF POLLINATION ON THE SUSCEPTIBILITY OF FLOWERS

Gossard and Walton (*10*) state that fertilized blossoms become resistant to infection by *Bacillus amylovorus* sooner than do unfertilized ones. Their experiments led them to conclude that blossoms which have been pollinated for 72 hours are not likely to be infected by the fire-blight organism and that susceptibility to infection does not

exist in blossoms that have been pollinated for 144 hours. They took the view that a host of bees by virtue of their pollinating activity would in reality be "friendly little sprites," transforming susceptible flowers into resistant ones by early pollination instead of being dreaded disseminators of blight as they are commonly supposed to be.

To gain further evidence of the possible influence of pollination on the susceptibility of flowers to infection by *Bacillus amylovorus*, studies were carried on in the field during the blooming seasons of 1926 and 1927. Inasmuch as practically all varieties of apples are self-sterile, it was not deemed necessary to emasculate the flowers, which was the procedure followed in Gossard and Walton's experiments. In the writer's experiments a number of flower clusters, both terminal and lateral, on several varieties of apples were inclosed in parchment paper bags before any of the blossoms had opened, so as to prevent natural pollination. When the blossom had opened, freshly collected pollen from other varieties was applied to the stigma of each flower by means of a camel's-hair brush. Other flowers were left unpollinated. At stated intervals after pollination inoculations were made by spraying pure broth cultures of *Bacillus amylovorus* into the receptacle cups by means of an atomizer. Unpollinated flowers were inoculated at the same time in like manner. The results of these studies are given in Table 12. While these data are not inclusive they suggest that the flowers remain susceptible to infection much longer after pollination than appeared from the experiments of Gossard and Walton.

TABLE 12.—*The influence of pollination on the susceptibility of flowers of some varieties of apples to infection by Bacillus amylovorus, Gays Mills, Wis., 1926*

Year and variety	Interval between pollination and inoculation	Pollinated clusters—		Unpollinated clusters—	
		Inoculated	Blighted	Inoculated	Blighted
1926	*Hours*	*Number*	*Per cent*	*Number*	*Per cent*
Dudley	69	6	100		
Do	114	6	100	10	100
Do	144	5	40	5	60
McMahon	70	4	100		
Do	122	2	100	4	100
Do	188	3	0		
Fameuse	50	8	100	5	100
Do	120	2	100	15	46
Do	122	7	71		
Do	168	5	0		
1927					
Northwestern Greening	191	16	62	9	88
Do	209	11	18	13	0
Do	216	11	0	11	0
McIntosh	118	4	100	4	0
Do	161	10	90	6	0
Do	213	10	0	12	0
Do	216	9	0	11	0
Fameuse	166	4	100		
Do	189	8	25	10	20
Do	211	3	0	9	0
Wealthy	187	5	100	4	0
Do	258	6	50		
Do	265	5	0		

DURATION OF SUSCEPTIBILITY OF YOUNG APPLE FRUITS

During the course of studies of the epidemiology of fire blight in the field in 1927 results were obtained which seemed to suggest that young apple fruits were susceptible to infection for some time after petal fall. (Fig. 2.) As experimental evidence seemed desirable, studies were conducted in the greenhouse in the spring of 1928, using potted dwarf Yellow Transparent apple trees. To prevent injury by aphids these trees had been sprayed with Derrisol (1–800) at intervals from the time the buds began to open until the blossoms opened. Pure suspensions of *Bacillus amylovorus* in sterile distilled water were sprayed at approximately daily intervals into the receptacle cups and upon the outer parts of successive series of young fruits which had previously been pollinated. The trees bearing the inoculated fruits were placed in the moist chamber for 24 hours after inoculation. They were incubated at 28° C. and 70 per cent humidity. The results of this study appear in Table 13. Field studies of the duration of susceptibility of young apple fruits to infection were also carried on in 1928. The young apples were sprayed with Derrisol (1–800) to kill any aphids which might be present, and at varying intervals after petal fall a pure suspension of *B. amylovorus* in sterile distilled water was sprayed into the receptacle cups and upon the outer parts of the young apples. However, as it was impossible to control the temperature and humidity following inoculation in the field, the results of these trials are not as striking as those obtained in the greenhouse. (Table 13.) While these studies need to be extended, the results indicate that, under favorable conditions, young apple fruits may remain susceptible to infection without the intervention of wounds for at least a week after petal fall.

TABLE 13.—*Results of inoculating young apple fruits, grown under conditions designed to prevent injury by insects or other agencies, with Bacillus amylovorus*[a] *at varying intervals after petal fall, 1928*

Location and variety inoculated	Number of days after petal fall on which inoculation was made	Fruits inoculated (number)	Fruits blighted	
			Number	Per cent
Greenhouse:				
Yellow Transparent	0	10	10	100
Do	2	30	30	100
Do	3	20	20	100
Do	4	9	8	89
Do	5	17	15	88
Do	6	20	15	75
Do	7	20	8	48
Do	10	11	10	90
Orchard:				
Fameuse	1	30	30	100
Do	2	21	21	100
Do	3	27	0	0
Do	4	29	0	0
Do	5	40	0	0
Do	6	30	0	0
Do	7	25	0	0
Do	10	36	0	0
Do	15	45	0	0

[a] The inoculum consisted of a pure suspension of *B. amylovorus* in sterile distilled water.

PRELIMINARY STUDIES ON THE NATURE OF RESISTANCE TO FIRE BLIGHT

Although the resistance of certain varieties of apples and pears to infection by *Bacillus amylovorous* has been noted by many investigators (Waite (*31*), Crandall(*8*), Jones (*17*), Whetzel and Stewart (*35*), Stewart (*28*), Hansen (*12*), Reimer (*22*, *23*), and others), comparatively little attention has been given to a consideration of the nature of this resistance. Hewitt (*15*), after a series of chemical analyses, concluded that an index to resistance and susceptibility is to be had in the ordinary analysis for starch. He states that those varieties which gave the higher percentages of starch upon analysis are more susceptible to injury by the blight organism. Stewart (*28*) attributes differences in varietal resistance to character of growth rather than to the presence of any inherent qualities which tend to make the trees immune. He says:

> The young tender tissue of the Douglass variety (a variety of pear which is presumably quite resistant to fire blight) is apparently as susceptible as that of any other pear but the tendency for the tissue to rapidly harden, soon after it is formed, impedes the activities of the blight bacteria and the injury is less severe.

Higgins (*16*) is of the opinion that the formation of gummy substances in the host tissues in response to bacterial invasion is responsible for resistance, as masses of gum were found in the border region between the living and dead tissues of a supposedly resistant variety of pear.

No variety of apple grown in Wisconsin has been found to be totally immune to fire-blight attacks. Even in varieties such as the Northwestern Greening and McIntosh, which commonly seem to be quite resistant, one occasionally finds a tree which is heavily infected. In 1927 several McIntosh trees on which more than 50 per cent of the flowers were diseased were found in the orchards at Gays Mills, although on the majority of the trees of this variety less than 3 per cent of the blossoms were blighted. (Table 11.) However, in those instances where blossom and twig blight were severe in trees of a resistant variety, it is a significant fact that in fully 95 per cent of the blighted twigs and diseased clusters examined only the current year's growth seemed to be invaded. Higgins (*16*) noticed a similar condition in the pear, for he reports that flower infection seems to occur as readily in resistant varieties as in the less resistant ones, but only a little damage is produced by each infection in the former. He states, furthermore, that in resistant varieties young twigs and flowers infected with fire blight are killed but "* * * infection in no case passed back into the mature wood of the previous season's growth."

In those cases where the pathogene had invaded the preceding year's growth the cankers formed on resistant varieties were quite superficial, the discoloration being limited in most instances to the cortex and the outermost part of the phloem. These cankers commonly cease enlarging soon after their formation and become delimited from the surrounding tissues by a peripheral cork barrier which gives a marked reaction with Sudan III. As the diseased tissue dries and shrinks a fissure usually develops along the boundary. In the susceptible varieties of apples, as the Fameuse and Transcendent, the organism does not commonly stop after reaching the older growth, but in most instances forms cankers or continues to invade the

branches, so that large limbs are often infected. In the cankers formed the discolored tissue, in a large number of instances, extends down to the cambial region. These cankers remain active for some time after their formation. It is significant that a much larger percentage of the cankers formed in the more susceptible varieties become holdovers than is the case in those formed in resistant varieties. (Table 1.) This difference in the degree to which invasion and subsequent inactivation take place in the resistant and the susceptible varieties seems to be correlated with the rapidity with which the host reacts to the invasion of the pathogene following infection. It would seem from these observations that the host offers specific resistance to the invasion of the pathogene in some instances and little or none in others.

STUDIES OF VARIABILITY IN RESISTANCE AND SUSCEPTIBILITY TO FIRE BLIGHT

In order to obtain more evidence relating to the nature of resistance to fire blight, a study of variability in varietal resistance or susceptibility to the disease was begun.

Relation of Temperature

Young shoots approximately 5 inches long grown on potted Wealthy (susceptible) and Northwestern Greening (fairly resistant) apple trees in the greenhouse were inoculated by inserting near the tip of the stem a needle which had been dipped in a pure broth culture of *Bacillus amylovorus*. Inoculated plants were then incubated in the chambers previously described (p. 596) at each of the following temperatures, 16°., 24°, and 28° C. The relative humidity at each of these temperatures was approximately 50 per cent. In the inoculated shoots which were incubated at 16° disease development was so slow in both varieties that manifestation of resistance or susceptibility was largely precluded. In each case only the apical portions of the shoots turned brown, there being no further extension of the disease down the stem. At 24° and 28°, however, the inoculated Wealthy shoots were completely blighted at the end of 10 days, whereas in the inoculated Northwestern Greening shoots the disease was limited to the apical parts of the twigs. In the inoculated Northwestern Greening shoots it was further observed that a stimulation of the buds in the axils of the remaining healthy leaves often took place and secondary shoots were produced. These observations would seem to indicate that within the range of conditions of this limited experimentation the resistance of the Northwestern Greening is not broken down or altered by variations in temperature.

Relations of Moisture

Young shoots grown on potted Wealthy and Northwestern Greening trees were inoculated by inserting near the tip of the stem a needle dipped in a pure broth culture of *Bacillus amylovorus*. They were then placed in a saturated atmosphere in an infection chamber.[7] The temperature in this chamber was maintained at 26° C. Under these conditions the inoculated shoots of the Northwestern Greening were just as severely attacked as the Wealthy. At the end of five

[7] This apparatus is described by Keitt and Jones (*18*).

days the inoculated Northwestern Greening twigs were completely blighted. Though it has not yet been feasible to conduct experiments concerning the possible influence of other factors, such as light and the type of vegetative growth of the experimental plants, it would appear probable from these results that resistance can be altered by subjecting so-called resistant varieties to the influence of humidity under suitable conditions of temperature and light. The reasons for this change in resistance to fire blight have not been fully analyzed as yet. However, a constantly maintained excess of water in the tissues would be expected to hinder the accumulation, oxidation, and condensation processes which are involved in the formation of the corky barrier which appears to limit the development of blight lesions under field conditions. Furthermore, it seems probable that an increase in the amount of moisture in the intercellular spaces would favor the growth and migration of the blight bacteria.

STUDIES OF METHODS OF CONTROL

Fire blight is one of the most difficult to combat of all of the diseases of pomaceous fruits. Arthur (*3*) suggested the cutting out of blighted parts as a control measure. Waite (*33*) and Smith (*24*) demonstrated that the excision method could be successfully employed if a sufficiently large area of the infested territory is adequately policed and the affected parts of the trees are promptly removed. Other methods of control have since been advocated, so that at present the control measures which are in vogue fall chiefly into five categories, as follows: (1) Ridding the trees of the sources of inoculum by excision methods, (2) control or elimination of insect carriers, (3) modification of the susceptibility of the host by appropriate cultural conditions, (4) spraying with Bordeaux mixture to reduce the number of blossom infections, and (5) the use of resistant varieties. In Wisconsin numerous attempts to control fire blight by the use of the commonly recommended methods have met with but indifferent success. The ineffectiveness of these measures in controlling the disease is particularly noticeable in years when epidemic outbreaks of fire blight occur. In view of the difficulties which have been encountered by the apple growers of this State in controlling fire blight it was deemed advisable to test experimentally the control measures now advocated and to seek to develop improved methods. Accordingly, several series of plots were marked out in the fall of 1925 at Gays Mills, Wis. and each plot was subjected to a special treatment. One plot was left untreated as a control. Counts were made after the blooming periods to determine the percentage of blossom blight developing in the different plots. Similar experiments were conducted in 1927 and 1928. The treatments and the results are shown in Table 14.

It is recognized that these experiments are not extensive enough to warrant definite conclusions, particularly in view of the small amount of disease which developed in most of the control plots. However, from these limited data the following conclusions seem to be warranted: (1) Excision seems to have led to some reduction in the amount of blight in the following season, but (2) spraying for the control of aphids did not result in any material reduction in the amount of blight. However, while excision reduced the number of infections in most of these plots, it has not fully controlled the disease. The infections in

the plots in which excision was practiced could be traced back, in most cases, to overwintering cankers and blighted twigs overlooked in the excision process. However, the writer exercised unusual care in the effort to accomplish thorough excision. It is scarcely to be expected that better results would be obtained in commerical orchard operations.

TABLE 14.—*Studies of the comparative value of various measures for the control of apple fire blight, Gays Mills, Wis., 1926–1928*

Year and series	Plot number	Variety	Treatment	Blossom clusters	
				Examined	Blighted
				Number	*Per cent*
1926					
Series I	1	Fameuse	Excision, September, 1925, and April, 1926; Scalecide (1-15), Apr. 30, 1926; Nicotine dust, May 3, 1926; Bordeaux, 2-4-50, June 23, 1926	3,394	*14
	2	do	Excision, September, 1925, and April, 1926	3,110	6
	3	do	Scalecide (1-15), Apr. 30, 1926; Nicotine dust, May 3, 1826; Bordeaux, 2-4-50, June 23, 1926	2,755	16
	4	do	Control	5,551	33
Series II	1	Wealthy	Excision, September, 1925; Scalecide (1-100), May 5, 1926	8,044	8
	2	do	Excision, September, 1925	8,672	5
	3	do	Scalecide (1-100), May 5, 1923	9,762	7
	4	do	Control	6,944	6
1927					
Series I	1	Fameuse	Excision, August and September, 1926; Nicotine dust, May 1, 1927	4,990	3
	2	do	Excision, August and September, 1926	4,022	4
	3	do	Nicotine dust, May 1, 1927	3,684	6
	4	do	Control	4,678	5
Series II	1	do	Excision, August, 1926	4,682	2
	2	do	Control	4,287	7
Series III	1	do	Excision, August. 1926	3,127	1
	2	do	Nicotine dust, May 1, 1927	1,730	0
	3	do	Control	3,155	3
Series IV	1	Wealthy	Nicotine sulphate (1-800), April, 1927	4,037	8
	2	do	Excision, August, 1926; Nicotine sulphate (1-800), April, 1927	4,523	12
	3	do	Control	4,403	14
1928					
Series I	1	Fameuse	Excision, August, 1927; Derrisol (1-800), Apr. 27, 1928	2,045	1
	2	do	Excision, August, 1927	2,160	2
	3	do	Derrisol (1-800), Apr. 27, 1928	2,974	5
	4	do	Control	1,000	2
Series II	1	do	Excision, August, 1927; Derrisol (1-800), Apr. 27, 1927	2,475	0
	2	do	Excision, August, 1927	2,530	0
	3	do	Derrisol (1-800), Apr. 27, 1928	2,260	2
	4	do	Control	3,300	2
Series III	1	do	Excision, August, 1927	1,915	1
	2	do	Control	1,925	1

* The unusually high blossom-blight count in this plot was due to a number of overwintering cankers which were overlooked in certain of the trees during excision.

BORDEAUX MIXTURE

Stevens, Ruth, and Peltier (*26*) and McCue (*20*) report that Bordeaux mixture applied during the blossoming period of pears seems to offer some protection against blossom blight. Reimer (*22, 23*) concluded after a series of experiments that Bordeaux mixture applied to pear trees during the open-cluster stage of blossom-bud development will materially reduce the number of blight infections but will not prevent all infections. Recently McCown (*19*) reports having re-

duced the number of blossom infections in apple by an application of Bordeaux mixture when the flowers were just coming into full bloom. In view of the fact that the inoculum for fire blight is largely rain-borne under Wisconsin conditions and that the critical period for infection includes the blossoming time, it was deemed advisable to determine whether an application of Bordeaux mixture at blossoming time would reduce the amount of infection in apples under local conditions. In 1926 Bordeaux mixture, 2–4–50, was applied to 40 Wealthy apple trees and 100 McMahons at full bloom, care being taken, in so far as it was possible, to cover the trees well. A month later blossom-blight counts were made in the sprayed and unsprayed plots. (Table 15.) In 1927 tests were again made, using a stronger solution of Bordeaux mixture in accordance with Reimer's (*22*, *23*) recommendations. In this experiment Bordeaux mixture, 3–6–50, was applied to 80 Fameuse trees when most of the blossoms were just beginning to open. The results, which appear in Table 15, show that the amount of blossom blight was not materially reduced by these Bordeaux-mixture applications. However, more data are needed before any valid conclusions can be drawn as to the value of Bordeaux-mixture applications in the control of blossom blight under Wisconsin conditions.

TABLE 15.—*The effect of Bordeaux-mixture applications on subsequent blossom-blight development, Gays Mills, Wis., 1926–27*

Year and variety	Strength of Bordeaux mixture used	Sprayed blossom clusters		Unsprayed blossom clusters	
		Examined	Blighted	Examined	Blighted
		Number	*Per cent*	*Number*	*Per cent*
1926					
Wealthy	2-4-50	7,170	2	7,045	5
McMahon	2-4-50	7,261	19	7,352	17
1927					
Fameuse	3-6-50	5,887	14	6,180	19

BACTERICIDES

In an attempt to find a suitable chemical treatment for the control of fireblight, laboratory and greenhouse tests were made. In the laboratory a number of different chemicals which were thought to possess bactericidal properties were tested. Among the substances tried were sodium silicofluoride, calcium silicofluoride, Bordeaux mixture, 4–4–50, Bordeaux mixture, 4–8–50, Uspulun,[8] Semesan Bel,[9] and Improved Semesan Bel.[10] In testing the bactericidal action of these substances the method outlined by Anderson and McClintic (*1*), with some modifications, was followed. Very briefly, the procedure used was as follows: One-tenth of a cubic centimeter of a 24-hour-old nutrient broth culture of *Bacillus amylovorus* was transferred by means of a sterile pipette to a test tube containing 5 c. c. of a solution of suspension of the chemical to be tested. This tube was

[8] A proprietary compound containing chlorophenol mercury.
[9] A proprietary compound containing hydroxymercurichlorophenol.
[10] An organic mercury compound.

shaken thoroughly and at the end of 2½, 5, and 10 minutes, respectively, a loopful (4 mm. in diameter) of the baterial suspension in the solution being tested was transferred to a test tube containing 10 c. c. of sterile nutrient broth. The tubes were then incubated at 24° C. for 48 hours. Growth in the tubes indicated that the substances at the particular concentration used were ineffective in killing the bacteria. A duplicate set of controls was run for each chemical tested. As controls, one-tenth of a cubic centimeter of a 24-hour-old nutrient broth culture of *B. amylovorus* was added to 5 c. c. of sterile nutrient broth. A loopful of the resulting bacterial suspension was transferred to a sterile tube of nutrient broth at 2½, 5, and 10 minute intervals, respectively. Of the substances tested, sodium silicofluoride and calcium silicofluoride failed to show significant bactericidal action against *B. amylovorus*, as they were ineffective at all dilutions tried. (Table 16.) Bordeaux mixture, 4–4–50 and 4–6–50, were also ineffective. A 1 per cent solution each of Uspulun and Semesan Bel, a one-sixteenth per cent solution of Improved Semesan Bel, and Bordeaux mixture, 4–8–50, were all effective in inactivating the bacteria, as was indicated by the lack of growth in the tubes of broth. The control tubes on the other hand, were heavily clouded, indicating that in these tubes growth had taken place. The most promising of these chemicals were subsequently subjected to further tests. In these tests approximately one-tenth cubic centimeter of each of a one-half per cent solution of Improved Semesan Bel and Bordeaux mixture 4–8–50 was placed on a sterile glass slide and allowed to air dry under aseptic conditions.

TABLE 16.—*The bactericidal action of certain chemicals on Bacillus amylovorus Madison, Wis., 1928*

Chemical and concentration [a]	Results [b] after exposure for—			Chemical and concentration [a]	Results [b] after exposure for—		
	2½ minutes	5 minutes	10 minutes		2½ minutes	5 minutes	10 minutes
Sodium silicofluoride:				Calcium carbonate:			
1:20,000	+	+	+	1:10	+	+	+
1:1,000	+	+	+	Uspulun:			
1:100	+	+	+	1:200	+	+	−
Calcium silicofluoride:				1:100	−	−	−
1:1,000	+	+	+	Semesan Bel:			
1:200		+	+	1:500	+	+	+
1:100		+	+	1:200	+	−	−
Bordeaux mixture:				1:100	−	−	−
4–6–50	+	+	−	Improved Semesan Bel:			
4–8–50	−	−	−	1:800	−	−	−
Calcium hydroxide:				1:1,600	−	−	−
1:10	+	−	−				

[a] The chemicals used, except proprietary products, were "chemically pure"; dilutions were made with sterile distilled water on the basis of weight.
[b] + = growth in the tubes of broth; − = no growth in the broth

Approximately five-hundredths of a cubic centimeter of a pure suspension of *B. amylovorus* in sterile distilled water was then added to the surface of the dried film on the slide. At the end of 10 minutes' exposure a loopful of the bacterial suspension was transferred to several drops of sterile water in a sterile Petri dish to which cornmeal agar was added and mixed with the bacterial suspension by the

usual poured-plate technic. At the same time a loopful of the bacterial suspension from a control was transferred to (1) a Petri dish containing sterile water and to (2) a Petri dish containing a loopful of the sterile bactericide. After a suitable incubation period the number of colonies in each plate was determined. This experiment was repeated several times with consistent results, a typical series of which appears in Table 17. These data show that the highly alkaline Bordeaux mixture exercised a strongly toxic effect (fig. 16, B) on the blight bacteria, while Improved Semesan Bel, 1–200, (fig. 16, A) completely inactivated them under the conditions of these experiments.

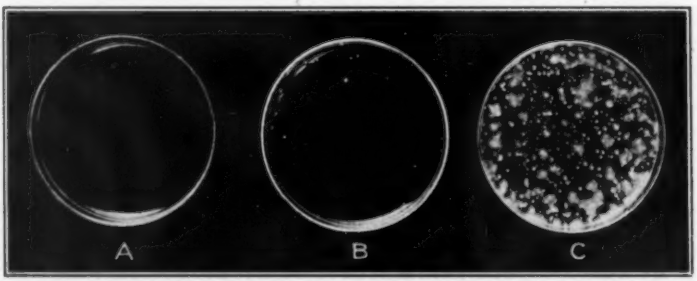

FIGURE 16.—Cultures showing the action of certain chemicals on *Bacillus amylovorus*: A, Bacteria exposed to the action of Improved Semesan Bel, one-half per cent, before plating; B, bacteria exposed to action of Bordeaux mixture, 4–8–50, before plating; C, control

TABLE 17.—*The toxic effect of certain bactericides* [a] *on Bacillus amylovorus*

Chemicals and concentration [b]	Average number of colonies per plate after 10 minutes exposure
Bordeaux mixture, 4–8–50	[c] 45
Improved Semesan Bel, 1:200	0
Untreated control	[d] +

[a] These bactericides were placed on sterile glass slides and allowed to become air dry under asceptic conditions; their action upon *B. amylovorus* was then tested.
[b] Dilutions were made with sterile distilled water on the basis of weight.
[c] The colonies which developed were mostly small, submerged, and somewhat abnormal in appearance.
[d] Colonies too numerous to count.

Tests of the effectiveness of Semesan Bel in reducing the amount of fire blight under field conditions were conducted in the spring of 1927. The limbs and trunks of 16 Fameuse apple trees which contained numerous hold-over cankers and twigs were thoroughly sprayed with a 2 per cent solution of Semesan Bel on April 20, when the blossom buds were in the early closed-cluster stage of development, and again five days later. No further applications were made, due to the limited amounts of the spray material on hand. Blossom-blight counts made later in the season failed to show any significant difference in the development of the disease on the sprayed and the unsprayed trees. (Table 18.) In 1928 further tests were made, using Improved Semesan Bel and several other organic mercury

compounds. In some instances as many as five applications were made, starting when the buds were in the early closed-cluster stage of development and continuing at intervals until petal fall. In some cases (Table 18) it would seem that the number of blight infections was reduced by certain of these chemicals. However, in interpreting these data it should be remembered that the number of overwintering sources of primary inoculum varied greatly in different trees. There seems to be considerable promise, nevertheless, in some of the chemicals tried. More data are necessary before conclusions can be drawn.

TABLE 18.—*Effect of certain chemical treatments on subsequent blight development on Fameuse apple, Gays Mills, Wis., 1927 and 1928*

Year and number of trees in plot	Treatment used	Dates of application	Stage of bud development [a]	Sprayed blossom clusters		Unsprayed blossom clusters	
				Total	Blighted	Total	Blighted
				Number	*Per cent*	*Number*	*Per cent*
1927							
16	Semesan Bel, 2 per cent	Apr. 20	GT	862	20	1,053	15
		Apr. 25	ECC				
1928							
10	Semesan Bel, ½ per cent	Apr. 21	GT	1,010	2	1,000	8
		do	GT				
		Apr. 26	ECC				
20	do	May 6	MCC	1,010	8	1,000	7
		May 11	OC				
		May 19	PF				
		May 6	MCC				
12	Semesan Bel, 1 per cent	May 11	OC	1,020	4	1,000	8
		May 19	PF				
		May 1	ECC				
		May 6	MCC				
4	K-1-GB,[b] 1 per cent	May 11	OC	385	6	618	3
		May 19	PF				
8	K-1-CB, 1 per cent	May 3	ECC	525	3	1,578	9
		May 6	MCC				
8	K-1-FB,1 per cent	May 3	ECC	745	1	1,578	9
		May 6	MCC				

[a] GT=Green tip; ECC=early closed cluster; MCC=middle closed cluster; OC=open cluster; PF=petal fall.
[b] K-1-GB, K-1-CB, and K-1-FB are organic mercury compounds.

SUMMARY

In Wisconsin, epidemic outbreaks of fire blight of apple are frequent and very destructive. Numerous attempts of orchardists to control the disease by the commonly recommended methods have been attended with but indifferent success. The variability in the severity of occurrence of fire blight and the difficulty of its control have led to further studies of the disease under local conditions. Two correlated lines of work have been undertaken: (1) Studies of the development and control of the disease in relation to the natural environment and (2) detailed studies, under partly controlled conditions in the laboratory and greenhouse, of certain phases of the life history of the casual organism in relation to pathogenesis.

Records of the seasonal development of the host, the pathogene, and the disease at Gays Mills, Wis., are presented for the years 1926, 1927, and 1928 in correlation with certain meteorological data. Although no general epidemic outbreaks of fire blight occurred in these years, local outbreaks were prevalent. These outbreaks were traceable to local sources of primary inoculum and appeared to be corre-

lated more with the amount and distribution of rainfall and suitable temperatures for infection than with insect activity.

Bacillus amylovorus was found to overwinter in the apple in Wisconsin in association with cankers and blighted twigs. While only a small percentage of the cankers or blighted twigs formed in any one year carried the blight organisms over the winter, it was shown that the bacteria live over winter under Wisconsin conditions in sufficient quantity to furnish a plentiful source of inoculum for primary infection. Varieties were found to differ greatly in the percentage of blighted twigs and of cankers which carry the blight bacteria through the winter. Of the varieties studied, the percentage of cankers in which the organism passed the winter was greatest in Transcendent, Yellow Transparent, Fameuse, and Wealthy. On the Dudley, McIntosh, and Northwestern Greening, on the the other hand, comparatively few hold-over lesions were found. The smooth-margined type of lesion was largely responsible for carrying the disease over winter. The delimited lesion was commonly found to be inactive.

Isolation experiments furnished further evidence that the blight organisms overwinter largely in the apparently healthy tissues immediately adjacent to the discolored margin of the canker. As 33 attempts made to isolate the bacteria from normal tissues 2 inches from the macroscopic margins of cankers were all negative, it appears unlikely that the blight bacteria often overwinter more than 1 inch from the visible margin of the canker.

Meteoric water was found to be the most important agent concerned in Wisconsin in the spread of the primary inoculum from hold-over cankers and twigs where the pathogene overwinters to the current year's growth.[11] Observations have failed to indicate that pollinating or other insects are concerned to any significant extent in the spread of the bacteria from overwintering sources to the susceptible parts.

Meteoric water and insects appear to be the most important agents, under Wisconsin conditions, for disseminating the secondary inoculum. In some instances, meteoric water seems to be an even more important agent than insects in the spread of the secondary inoculum for both blossom blight and twig blight.

Laboratory and greenhouse studies of the mode of entry of *Bacillus amylovorus* indicate that, under favorable conditions, the fire blight pathogene can gain access to the tissues of the host through stomata. Cases of stomatal penetration have been found in inoculated tissues taken from (1) young apple leaves, (2) sepals of unopened apple blossoms, and (3) the inside of the receptacle cups of apple and pear flowers that were open when inoculated. In each case the inoculation was made by atomizing with a pure suspension of *B. amylovorus* in water.

Studies of the migration of *Bacillus amylovorus* suggest that in the initial stages of invasion of the host tissues the fire-blight bacteria are free-swimming in the liquid in the intercellular spaces. In later stages of infection they were found embedded in a substance

[11] Since this manuscript was submitted Dr. E. C. Tullis has published certain studies on the overwintering and modes of infection of fire blight. (Mich. Agr. Expt. Sta. Tech. Bul. 97, 32 p. 1929.) In the main Doctor Tullis's conclusions are similar to those of the author.

which stains differently than do the bacteria. The nature of this substance was not definitely determined. However, conclusive evidence that such masses are true zoogloea seems to be lacking. In the advanced stages of the disease, *B. amylovorus* was found within the cells. In some instances the bacteria appeared to have migrated from one cell to another through apertures in the walls.

Studies of the parasitism of *Bacillus amylovorus* suggest that the host cells are probably killed by plasmolysis following the extraction of water from the cell contents. Toxin production was not demonstrated with the methods used. In a number of hold-over cankers which were examined histologically, the bacteria were found within the sieve tubes of the phloem in the area immediately surrounding the discolored margin of the canker. In several instances the bacteria were found so crowded in the sieve tube cells of the phloem that individual bacterial rods were distinguishable only with difficulty. No evidence of cyst formation was found.

All varieties of apples grown in Wisconsin are susceptible in a greater or less degree to fire blight. However, such varieties as the Transcendent, Yellow Transparent, Fameuse, MacMahon, and Wealthy are subject to severe attacks of fire blight, whereas the McIntosh, Dudley, and Northwestern Greening are seldom severely infected.

Preliminary studies of the nature of resistance to fire blight are reported. These studies indicate that resistance is probably due, in part at least, to the laying down of a corky barrier by the host. In some varieties this barrier seems to be laid down very quickly after infection.

Studies of variability in varietal resistance to fire blight are reported. Variations in temperature within the limited range used did not appear to have any significant influence on the expression of resistance to fire blight. Subjecting so-called resistant varieties to variations in moisture, however, did seem to affect resistance.

The results of studies of the influence of pollination on the susceptibility of flowers to infection by *Bacillus amylovorus* were not fully conclusive. They show, however, that apple flowers may remain susceptible to infection by *B. amylovorus* for at least 150 hours after pollination.

Control experiments conducted during 1926, 1927, and 1928 are reported. The value of the results is limited because of the small amount of disease which developed in these years. However, excision of blight lesions in the dormant period appeared to cause some reduction in the amount of blossom and twig blight in the following season. The control of aphids did not result in any significant diminution in the amount of blight. It should be borne in mind, however, that comparatively little twig blight occurred under the conditions of these experiments. Bordeaux misture, 3–6–50, applied at blossoming time afforded little protection from blossom blight of apples in 1926 and 1927. Experiments are reported in which an effort has been made to discover a suitable bactericidal treatment which would inactivate such inoculum as escapes the excision process. Thus far, the results from such studies are not conclusive.

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STUDIES OF QUALITY AND MATURITY OF APPLES[1]

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INTRODUCTION

The term maturity as used in discussing crop growth and harvest is indefinite in meaning. It does not define or locate an exact condition or date before which the material under discussion is immature, or at which it is mature, or after which it is overmature. Apples harvested extremely early are commonly of poor quality for eating, and if harvest is delayed until the crop is falling it is usually of poor storage quality. These facts form the basis of the problem discussed in this paper.

REVIEW OF LITERATURE

Magness and Diehl (*13*)[2] emphasize the fact that the ripening process in apples is a combination of many factors. The different changes that take place in the fruit as it approaches maturity on the tree may continue at varying rates in different seasons and under different climatic and nutritional conditions. Magness and Diehl (*12*) state that the general changes in apples while ripening on the tree are (1) changes in acidity, (2) changes in sugar and starch content, (3) changes in the pectin content, and (4) changes in the moisture content. Plagge, Maney, and Gerhardt (*17*) are of the opinion that some of the changes associated with the ripening of apples on the tree and in storage are a loss of moisture, acidity, dextrins, starch, and acid-hydrolyzable material. They found an increase in specific gravity, sugars, and soluble pectin.

Browne (*3*) states that the sweetness of an apple is dependent on the absence of malic acid rather than on an excess of sugar as is commonly supposed. Apples frequently contain more sugar than fruit of a sweeter kind. Magness and Burroughs (*11*) state that acidity is an important factor in the flavor of fruit and is largely responsible for its freshness of taste. Magness (*10*) finds that sugars and acids are intimately linked with fruit flavor. Magness and Diehl (*12*) believe that acidity is of much importance in determining the quality of fruit.

Bigelow, Gore, and Howard (*1*) recognize that the variation in composition of fruit on the tree introduces a possible variation that is exceedingly difficult to calculate. They feel that only by obtaining a large amount of evidence can a clear indication be obtained of the changes that occur. Jones and Colver (*8*) believe that the composition of apples may vary with their position on the tree. Magness

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[2] Reference is made by number (italic) to "Literature cited," p. 638.

and Diehl (*13*) note a wide variation in sugar content in apples of the same variety and from the same trees grown in Virginia during the two seasons 1923 and 1924. The 1924 crop was distinctly lower in sugar content.

Total sugars have been shown to increase through part or all of the growing season (*7, 18*), as well as during storage (*12, 13*). It is believed that an important change is that of starch to sugar. Probably certain pectin and hemicellulose compounds are converted into sugars (*11*). Starch has been shown to decrease during the growing and storage season (*1, 12*) and invert sugar has been found to increase during growth and storage (*1, 19, 15*). According to Thompson and Whittier (*19*), the reducing material consists mainly of levulose. Sucrose has been shown to increase during ripening and to decrease in storage (*15, 17*). Magness and Burroughs (*11*) find that sugar changes are much less affected by storage conditions than are acidity changes.

A decrease in acidity during growth has been noted by several workers (*1, 3, 17, 18*). Magness and Diehl (*12*) suggest that the most accurate conception of acidity changes in apples while on the tree is that of high acid content early in the season followed by a dilution as the fruit increases in size with a consequent low acid concentration in the mature fruit. Because of the wide variation in acidity in individual apples it would be necessary to use large samples to determine accurately what influences the rate of acidity loss.

Magness and Burroughs (*11*) find that in stored apples the acidity varies greatly with the conditions under which the fruit is stored, and Magness and Diehl (*13*) call attention to the fact that because of the wide variations existing particularly in the acid content of different individual apples, it is difficult to determine accurately the decrease in acidity under different storage conditions. Haynes's (*6*) acidity determinations on individual apples show that the average deviation from the mean was 6 to 15 per cent of the mean. Other determinations showed the percentage to vary from 13 to 19. Evans (*5*) finds a rather wide range in acidity and total sugars in samples of identical history and like degree of maturity.

Bigelow, Gore, and Howard (*1*) note that the chemical composition of apples changes very rapidly after they are picked from the trees. In six days the composition of summer apples changed from 24 per cent starch, 4.6 per cent sucrose, and 24 per cent invert sugar to 5.9 per cent starch, 12.9 per cent of sucrose, and 38.2 per cent sugar. This was a greater change in starch than took place in apples which remained on the tree for 43 days. Plagge, Maney, and Gerhardt (*17*) think the time of picking or condition of maturity at the time of storage within certain limits affected but little the ultimate chemical composition of the apples in cold storage. Under cold-storage conditions acidity, dextrin, starches, and acid-hydrolyzable material gradually decreased with a correspondingly slow increase in simple and disaccharide sugars.

MATERIALS AND METHODS

Jonathan apples from the experiment station orchard at Pullman, Wash., formed the basis of the larger part of the work reported in this

paper. Work was also done on Rome Beauty, Winesap, and Delicious from Yakima and on Jonathan and Delicious from Clarkston, Wash.

The data for 1923 and 1924 crops include measurements and analyses on samples from a period several weeks before the ordinary harvest date through harvest and through the ordinary period of storage. These samples were collected at intervals representing as nearly as possible definite stages in the maturity of the fruit. The fruits were hand picked from all parts of the trees and brought directly to the laboratory, where some were used by the horticultural division for the study of physical characteristics, including pressure tests as described by Morris (*14*), and for the recording of the histological data. The remainder were used for chemical analysis.

During the 1924 season, duplicate samples were collected to obtain data on the natural variation occurring between samples from the same tree or from the same orchard. Duplicate determinations were then made on each of the duplicate samples.

Six to eight representative apples were quartered and cored and a thin slice was taken from each of the various quarters until the required weight of sample was obtained.

To determine the dry weight 50 gm. of the sample were weighed into a tared beaker, evaporated down on the steam bath for a short time, placed in a vacuum oven at 70° C. and 15 cm. pressure for five hours or to constant weight.

Ash was determined on the sample remaining from the moisture determination by burning in a muffle furnace.

To determine the sugars and acid-hydrolyzable reducing material, 25-gm. samples were covered with 100 c. c. of 95 per cent alcohol, the acid was neutralized, and the sample boiled vigorously for a few minutes on the steam bath. The alcohol was carefully decanted and the residue extracted with alcohol for 12 hours. The extract was added to the original filtrate and made up to 500 c. c. with distilled water. Reducing substances were determined on 10 c. c. of this solution by the Munson and Walker method, weighing the cuprous oxide. Fifty cubic centimeters of the above solution were hydrolyzed by adding 10 c. c. of hydrocholoric acid, making up to 100 c. c., and allowing to stand overnight. Reducing substances were again determined and calculated as dextrose. The residue from the alcohol extract was dried and hydrolyzed with 125 c. c. 2.5 per cent hydrochloric acid under a reflux condenser for two and a half hours. The solution was cooled and nearly neutralized with sodium hydroxide. This was made up to 250 c. c. and reducing substances were determined on a 10 c. c. aliquot. This is referred to as alcohol-insoluble acid-hydrolyzable material.

For the determination of acid 50 gm. of the sample were placed in a beaker, 150 c. c. of distilled water were added, and the material immediately boiled. The solution was made up to 500 c. c. and allowed to stand with frequent shaking for three days, 2 c. c. of toluol having been added to prevent decomposition. One hundred cubic centimeters of this solution were then titrated with 0.1 N sodium hydroxide. The end point was determined by phenolphthalein as indicator.

A portion of the sample was ground through a hand mill and the juice expressed. The viscosity was determined at 25° C. Hydrogen-ion concentration was determined with a Hildebrand electrode, using a Leeds and Northrup type K potentiometer.

The acidity of the juice was determined by titrating 25 c. c. of the juice with 0.1 N sodium hydroxide, the end point being determined electrometrically. Titration curves were constructed.

Nitrogen was determined on 25 gm. of the sample by the ordinary Kjeldahl method.

RESULTS IN 1923

During the 1923 season Jonathan apples were collected from the station orchard on seven different dates extending from August 30, when the fruit was still decidedly green, to October 23, which was well past the commercial harvest date. The results obtained from the chemical analyses of these samples are presented in Table 1. The data are the averages of determinations on duplicate samples taken from individual orchard samples.

TABLE 1.—*Composition of Jonathan apples harvested on the Washington Experiment Station farm in 1923*

Date harvested	Dry matter	Ash	Acid as malic	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material	Remarks
					Reducing	Sucrose	Total		
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	
Aug. 30	------	0.280	0.91	0.060	8.77	1.87	10.64	3.72	Very green, very hard, 5 per cent red, sour, no aromatic flavor, poor eating quality.
Sept. 10	------	.269	.97	.065	8.40	2.14	10.54	3.66	Green, very hard, 8 per cent red, no aromatic flavor, poor eating quality.
Sept. 20	18.84	.345	.84	.058	8.50	2.72	11.22	3.72	Green, very hard, 20 per cent red, slight aromatic flavor, poor to medium eating quality.
Sept. 26	19.23	.286	.96	.050	8.89	3.25	12.14	3.67	Green, hard, 50 per cent red, distinct aromatic flavor, medium eating quality.
Oct. 9	17.18	.208	.83	.046	7.50	3.94	11.44	2.02	Green to yellowish green, hard, 75 per cent red, distinct aromatic flavor, good eating quality.
Oct. 16	19.26	.262	.78	.047	8.98	3.54	12.52	2.07	Greenish yellow, hard, 85 per cent red, high aromatic flavor, very good to best eating quality.
Oct. 23	17.97	.234	.73	.053	8.98	4.74	13.72	1.38	Greenish yellow, firm to hard, 90 per cent red, medium to high aromatic flavor, very good to best eating quality.

The data do not indicate any consistent or progressive variation in the percentage of dry matter through the growing season. The same is true of the ash and nitrogen, although it may be said that there seems to be a tendency for the nitrogen to decrease. The percentage of acid may be interpreted as showing a tendency to decrease as the apple matures, although in the light of the average variation in Table 5 this decrease is not significant since the actual amount of acid per apple increases slightly. The total sugars increased about 3 per cent from August 30 to October 23. This increase is almost entirely accounted for by the increase in sucrose, since the change in the amount of reducing sugars was small. Sucrose showed a decided increase during the 1923 season; on October 9 there had been an increase of about 110 per cent over the amount found on August 30. Two weeks later, on October 23, the total increase was over 150 per cent.

The question arises as to the source of this additional sucrose. In this connection it is interesting to study the variation in the

percentage of alcohol-insoluble acid-hydrolyzable materials (called starchy material and starch in Washington Bulletin 205 (*16*)). This portion of the apple undoubtedly includes pentosans, cellulose, hemicellulose, glucosides, pectoses, and other carbohydrate bodies besides starch that undergo hydrolysis and are converted into reducing substances on boiling with hydrochloric acid but can not be considered starch, as emphasized by Magness (*9*). These alcohol-insoluble acid-hydrolyzable materials decreased from about 3.7 to about 1.4 per cent, or a decrease of about 63 per cent.[3] During the same period the sucrose increased from 1.87 to 4.74 per cent, or an increase of more than 150 per cent. The weight of alcohol-insoluble acid-hydrolyzable material per apple increased up to September 26, after which there was a drop in the amount of this fraction. The weight of sucrose per apple steadily increased.

Three possible sources of sucrose are suggested. A part may come from the alcohol-insoluble acid-hydrolyzable portion, since this shows a percentage decrease and also a decrease in the amount per apple after a certain date. It is also possible that increased enzymatic activity may have converted a part of the alcohol-insoluble material, which is not hydrolyzable by hydrochloric acid, into sucrose. The principle part is, however, probably synthesized from hexoses, which are translocated from the leaves.

The percentage of the alcohol-insoluble acid-hydrolyzable fraction does not show a progressive decrease throughout the period of this study, but remains practically constant through September 26, and then drops very decidedly by the next picking date. There were no killing frosts during this interval. On the other hand, the quantity of sucrose shows a fairly regular progressive increase.

PHYSICAL-CHEMICAL DATA

In addition to the chemical data just discussed, certain physical-chemical measurements have been recorded and are presented in Table 2. These were made on the juice expressed from the apples. The results of the measurement of conductivity on the same water extract which was prepared for the determination of total acidity are also given.

TABLE 2.—*Physical constants of juice from Jonathan apples harvested in 1923*

Date harvested	Specific gravity	Viscosity	pH	Acidity (per cent)	Conductivity	Conductivity of water extract
Aug. 30	1.0539	1.76	2.84	1.02	39×10^{-4}	5.76×10^{-4}
Sept. 10	1.0514	1.71	2.77	.96	36.8×10^{-4}	6.22×10^{-4}
Sept. 20	1.0602	2.07	1.32	1.10	31.1×10^{-4}	6.22×10^{-4}
Sept. 26	1.0458	1.75	3.26	.75	31.8×10^{-4}	8.19×10^{-4}
Oct. 9	1.0621	1.89	3.13	.88	31.8×10^{-4}	5.68×10^{-4}
Oct. 16	1.0616	1.84	3.25	.73	29.5×10^{-4}	5.91×10^{-4}
Oct. 23	1.0613	1.78	3.35	.64	39.5×10^{-4}	5.88×10^{-4}

[3] In order more clearly to indicate the comparison with the sucrose increase of 150 per cent, it may be stated that these alcohol-insoluble acid-hydrolyzable materials were 170 per cent higher at the beginning than at the close of the period.

The measurements in these data which suggest a progressive variation are those of hydrogen-ion activity and acidity of the juice as measured by electrometric titration. There is a greater variation in the acidity of the juice than in the total acidity as determined on the water extract, and varying in the same direction is the hydrogen-ion activity, which is decreasing (increasing pH).

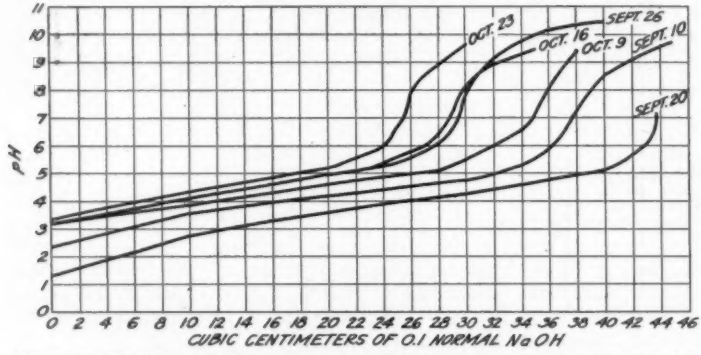

FIGURE 1.—Titration curves showing acidity of juice of apples harvested in 1923 on dates indicated

The titration curves for the juice are presented in Figure 1. These indicate graphically the variations in pH and in total acidity mentioned above, and in addition they show that the buffer value of the juice is about the same at the different stages of maturity.

TABLE 3.—*Changes in composition of Jonathan apples harvested October 5, 1923, and stored at 45° to 50° F.*

Date	Dry matter	Ash	Acid as malic	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material
					Reducing	Sucrose	Total	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Dec. 5	15.74	0.31	0.59	0.050	7.98	3.44	11.42	1.07
Dec. 7	17.19	.232	----------	.052	8.70	3.86	12.56	.89
Jan. 3	17.10	.11	----------	.046	8.94	3.66	12.60	.79

STORAGE RESULTS

The data obtained on the Jonathan apples from the station farm which were placed in storage are presented in Table 3. These apples were harvested and stored at 45° to 50° F. on October 5.

Apples which varied in size were selected in an effort to determine whether the composition varied with size and also whether size were correlated with keeping quality, for Morris (*14*) had found that large fruits of the same variety, from the same tree, and harvested at the same time, do not keep as well as smaller fruits. The data in Table 3 do not suggest such variation. A comparison of these data with those for the fruit picked from the trees (Table 1) shows that a further decrease of acid and of the alcohol-insoluble acid-hydrolyzable material takes place after the fruit is placed in storage.

RESULTS IN 1924[4]

In 1924 the work was enlarged to include the analysis of duplicate samples. As previously stated, duplicate determinations were made on all samples analyzed in 1923. In 1924 a sample was collected from a section of the orchard to be studied, care being exercised to make the sample as representative as possible from the horticultural and chemical standpoint. The same trees were sampled a second, and, in several cases, a third time. Duplicate determinations of each of the fractions studied were made on each of the duplicate or triplicate samples.

The data obtained on the samples collected during the growing season of 1924 are presented in Table 4 and Figure 2.

The figures in Table 4 represent the average of the duplicate and triplicate determinations. Comparing the variation between the amount of dry matter in duplicate and triplicate samples shown in Table 4 and that for storage apples shown in Table 6, it was found

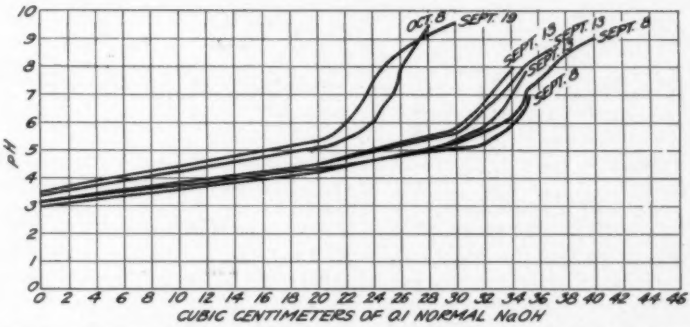

FIGURE 2.—Titration curves showing acidity of juice of apples harvested in 1924 on dates indicated

that the average variation between the duplicate or triplicate samples was 0.684. (Table 5.) The average variations for other determinations are also shown in Table 5.

The data for the 1924 storage apples are given in Table 6. These apples were harvested and placed in storage at 45° to 50° F. on September 25.

Tables 4 and 6 also give the averages of the analyses of duplicate samples. If we assume that a variation three times the average variation is a significant variation it would appear that the variation in dry matter is not significant during the growing season, since the variation between the earliest and latest picking is only 0.82 per cent and no intermediate variation is equal to more than twice the average variation. Viewed in the same way it is doubtful if there is a significant variation in dry matter during storage.

Considering the total acid, it again appears that the largest variation is less than twice the average variation. The data for the amount of acid in storage might be interpreted by inspection to show a decrease. However, it is only twice the average variation.

[4] The writers desire to express their thanks to Tom Beal for assistance in the analytical work of 1924.

TABLE 4.—*Composition of duplicate and triplicate samples of Jonathan apples harvested on the Washington experiment station farm in 1924*

Date of harvest	Dry matter	Ash	Acid as malic	Acidity of juice	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material	Remarks
						Reducing	Sucrose	Total		
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
Sept. 1	17.40	0.278	0.868	.....	0.0625	7.19	1.87	9.06	3.62	Very green, very hard, 5 per cent red, no aroma, very poor eating quality.
	17.30	.327	.912	.....	.058	6.85	1.98	8.83	3.70	
Average	17.35	.303	.890	.....	.0603	7.02	1.93	8.95	3.66	
Sept. 8	17.39	.312	.877	0.819	.059	6.52	3.46	9.98	3.35	Very green, very hard, 12 per cent red, very slight aromatic flavor, very sour, low to medium eating quality.
	[a]18.47	.255	.854	.765	.047	7.15	3.37	10.52	3.53	
	[b]18.56	.222	.820	.760	.046	6.84	2.79	9.63	3.40	
Average	18.14	.263	.850	.....	.051	6.84	3.21	10.04	3.43	
Sept. 13	[b]16.85	.322	.872	.856	.052	6.27	2.89	9.14	2.16	Very green, very hard, 16 per cent red, slight aromatic flavor, poor to medium eating quality.
	[a]16.78	.290	.850	.785	.050	6.94	2.49	9.43	2.40	
	17.27	.319	.861	.800	.045	6.53	2.39	8.92	2.15	
Average	16.97	.310	.861	.....	.049	6.58	2.59	9.16	2.24	
Sept. 19	16.57	.306	.819	.....	.058	6.74	1.54	8.28	1.76	Green, hard, 35 per cent red, slight aromatic flavor, medium eating quality.
	15.27	.....	.607	.....	.040	.....	.....	7.86	1.30	
	15.82	.238	.682	.....	.040	.....	.....	7.34	1.53	
Average	15.89	.272	.703	.....	.046	6.74	1.54	7.83	1.53	
Oct. 8	16.10	.223	.734	.....	.034	7.23	3.93	11.16	1.29	Yellowish green, hard, 60 per cent red, distinct aromatic flavor, medium to good eating quality.
	16.95	.226	.....	.....	.038	7.54	3.34	10.88	1.29	
Average	16.53	.225	.734	.....	.036	7.39	2.64	11.02	1.29	

[a] South side of tree. [b] North side of tree.

TABLE 5.—*Average variations between duplicate samples calculated on 1924 data*

	Average variation in—					
	Dry matter	Total acid as malic	Sugar			Alcohol-insoluble acid-hydrolyzable material
			Reducing	Sucrose	Total	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Average variation	0.684	0.083	0.346	0.478	0.571	0.159

TABLE 6.—*Changes in composition of duplicate and triplicate samples of Jonathan apples harvested September 25, 1924, and stored at 45° to 50° F. until dates indicated*

Date	Dry matter	Ash	Acid as malic	Nitrogen	Sugar			Alcohol-insoluble acid-hydrolyzable material
					Reducing	Sucrose	Total	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Oct. 27	16.44	0.294	0.586	0.037	7.41	4.71	12.12	0.65
Nov. 4	16.57	.307	.547	.037	7.42	3.99	11.41	.75
	16.96	.238	.572	.040	7.48	4.84	12.32	.71
Average	16.77	.273	.560	.038	7.45	4.42	11.87	.73
Dec. 10	14.73	.303	.500	.052	7.41	3.37	10.78	.66
	16.50	.236	.552	.046	9.02	3.18	12.20	.83
Average	15.62	.270	.526	.049	8.22	3.27	11.49	.74
Jan. 7	15.21	.230	.418	.044	8.54	2.20	10.74	.71

Unfortunately, we do not have a determination of reducing sugar for the samples taken on September 19. The variation from September 1 to 13 is a trifle more than the average variation between duplicate samples. From September 13 to October 8 the reducing sugars increased 0.81 per cent, or more than twice the average variation. In storage on October 27 and November 4 these sugars were practically the same as at the last picking. Over a month later, on December 10, there had been an increase of 0.77 per cent of reducing sugars, which is a trifle over twice the average variation.

The variation between samples in the amount of total sugars is large, as shown by comparison with the average variation. During the growing season the variation is first in one direction and then in the other. On the whole, however, it would appear that there is an increase in total sugars, since the difference between the amount found for October 8 and for each of the previous dates is either practically as great as or greater than three times the average variation for total sugars. It also appears that there was a further slight increase in total sugar for three weeks after the apples were placed in storage. Following this the total sugars appear to have decreased, although this, too, is open to question, due again to the fact that the January 7 analysis was on one sample only.

During growth the changes in sucrose followed in general the changes in total sugars. For the first three weeks in storage there was an increase in sucrose after which there was a decrease, the decrease being greater than that in total sugars. This was due, of course, to the increase in reducing sugars and indicates that sucrose is converted into reducing sugars during storage.

The alcohol-insoluble acid-hydrolyzable material probably shows a greater variation than any of the other fractions determined. This will be further discussed when a comparison of the three years' results is made. This material shows a progressive decrease through the growing period. The total decrease from September 1 to October 8 is nearly fifteen times the average variation. There was a further significant drop in this fraction during the first three weeks of storage, after which it remained practically constant.

COMPARISON OF RESULTS OF 1923, 1924, AND 1925 [8]

Considering the data for the three years as a whole, it appears that there is a fluctuation in dry matter but no significant variation. In ash and nitrogen there is likewise little variation. The figures for total acid are probably the most difficult to interpret, but on the basis of the average variation, as discussed above, it appears that there is no significant variation. However, considering the figures, there appears to be a perceptible decrease in total acid, although this decrease is very small in many cases, and in no case is the decrease for the growing season equal to much more than twice the average variation. It would therefore seem best to conclude that there is no definite evidence of a significant decrease as the season advances. The only way in which it can definitely be shown whether or not the acid decreases is to analyze a much larger number of samples. The writers' data agree with those of other workers in showing a perceptible decrease in acid as the fruit ages, but the significance of this

[8] See reference (*16*) for 1925 data.

decrease may have been overemphasized. The data for all three years agree in showing an increase in total sugars during the growing season. It is also apparent that this increase is due largely to an increase in sucrose, since there is no progressive decrease in the amount of this material as the season advances.

Of the total sugars it appears that there were appreciably more in the apples in 1923 than in either 1924 or 1925, which showed approximately the same amount. The same is true of the reducing sugars. The amount of sucrose seems to have been about the same for the three years. The amount of alcohol-insoluble acid-hydrolyzable material seems to have been about the same for 1923 and 1924, although the bulk of the decrease in this material came earlier in 1924 than in 1923. This was probably due to the fact that the 1924 harvest season was earlier. The amount of this fraction in the 1925 crop was decidedly less throughout the season than in the other two years, as is readily apparent from Table 7.

TABLE 7.—*Percentage of alcohol-insoluble acid-hydrolyzable material in Jonathan apples harvested in 1923, 1924, and 1925*

1923		1924		1925	
Date harvested	Per cent	Date harvested	Per cent	Date harvested	Per cent
Aug. 30	3.72	Sept. 1	3.66	Sept. 2	2.13
Sept. 10	3.66	Sept. 8	3.43	Sept. 9	1.49
Sept. 20	3.72	Sept. 13	2.24	Sept. 16	1.17
Sept. 26	3.67	Sept. 19	1.53	Sept. 23	1.40
Oct. 9	2.02	Oct. 8	1.29	Sept. 30	1.13
Oct. 16	2.07			Oct. 7	.82
Oct. 23	1.38				

From these data it would not seem possible to establish a definite level to which this fraction must come in order to indicate a desirable period of maturity, but it is more than probable that it will be necessary to follow the amount of this material through the season to a point where there is a decided drop in the amount. Further work such as was suggested in 1924 is apparently necessary to establish this point.

An interpretation by inspection would suggest that the amount of acid found in the 1925 crop is somewhat less than that in the two preceding crops. This difference is, however, only approximately twice the average variation.

The annual rainfall at Pullman is about 20 to 22 inches. A very small proportion of it falls during the summer. The college orchard is not irrigated, and the soil is usually quite dry during August and September. The apples are small to medium in size, but when harvested late are of good color and of good eating quality. Those of the crop of 1923 were well colored and very small, but continued to increase in size until after the last samples were harvested. The fruits of the 1924 crop were very much larger than those of the 1923 crop, were well colored, and continued to increase in size until after the middle of October.

The season of 1923 was practically without rainfall during August and September. Bright, clear weather prevailed until October 16 with relatively high temperature for the district. August 26 to 31, inclusive, had an average daily maximum temperature of 88° F. and

an average daily sunshine of 12 hours. September had an average daily maximum temperature of 80° and an average daily sunshine of approximately 8 hours. October 1 to 23, inclusive, had an average daily maximum temperature of 57° and an average daily sunshine of 4 hours.

In 1924 there were several light rains during the latter part of August and during September and October. These fell as slow drizzles, and a high atmospheric humidity prevailed during the cloudy days that followed. August 26 to 31 had an average daily maximum temperature of 66° F. and an average daily sunshine of 9½ hours. September had an average daily maximum temperature of 61°, and an average daily sunshine of approximately 8 hours. The first eight days of October had an average daily maximum temperature of 49°, and an average daily sunshine of approximately 4 hours.

The 1923 season was thus characterized by a large amount of sunshine, relatively high temperatures, and little or no rain, while that of 1924 was characterized by light rains, less sunshine, and lower temperatures. As previously suggested, it would therefore be expected that the fruit harvested in 1923 would be of better quality than that obtained in 1924. By reference to Tables 1 and 4 and accompanying legends this is found to be the case, as indicated by the amount of sugars present and by observations on color, flavor, and eating quality.

QUALITY OF APPLES AS RELATED TO GROWTH AND EXPOSURE

Tests of the eating quality of fruit were made of each crop of apples from 1923 to 1926, inclusive. These studies were confined to Jonathan, Rome Beauty, Winesap, and Delicious. A great variation was found in the quality of fruit within a variety and also in the quality of fruit from a single tree. Trees that suffered from drought during the middle and latter part of the summer usually produced fruit of very low quality. This is the first fact made clear in these tests. The similarity of low quality in some fruits from trees that seemed to be well supplied with water to those that were evidently suffering from lack of water, led to a careful study of the effects of environment on tree growth, and of weather on the quality of individual fruits. In this study quality was determined by taste. The fruits were graded as excellent, good, medium, and poor according to the ratings for quality given by 109 persons who tasted the fruits. In 1925 and 1926 the quality of large numbers of fruits growing under the environmental conditions indicated in Table 10 was determined at harvest and after 30 and 90 days of storage.

Dastur (*4*) in a study of the relation of water content to photosynthesis found that a close relationship exists between the rate of assimilation of food by the plant and the water content of the foliage.

Many tests to determine the loss of water by the fruit were carried out. The results of one of these made in late August are presented here. Twenty fruiting spurs with fruit attached were removed from the tree, taken to the laboratory, and weighed before 8 a. m. Ten of these were left with the fruit and foliage attached. The fruit of the other 10 was separated from the twig. The lots were weighed and left on tables in the laboratory fully exposed to the dry air at a temperature of 78° F. The results are given in Table 8.

TABLE 8.—*Loss of water by apple fruit and foliage when left exposed to dry air on a laboratory table at 78° F.*

Item	Average weight at—		Weight loss
	8 a. m.	5 p. m.	
	Grams	*Grams*	*Grams*
Apples with twig and foliage attached	159.2	152.1	7.1
Apples with twig and foliage detached	145.5	145.1	.4
Twigs and foliage only of second lot above	11.2	7.0	4.2

Apples attached to the twigs carrying the foliage became very much wilted and showed shriveling of the skin while the detached fruit remained apparently plump and was only slightly wilted. Fruits attached to the largest amount of foliage lost weight most rapidly, while those attached to a few leaves only lost weight more slowly and the foliage remained green for a longer time.

Apples on trees carrying a heavy load of foliage and a medium to light crop of fruit are often noticeably wilted during the very hot weather of summer. This is most apparent on fruit attached to twigs carrying a large amount of foliage and on varieties that have very large leaves. Water evaporates from the foliage more rapidly than it can be supplied by the tree and the twig draws some direct from the apples.

Brooks and Fisher (*2*) showed that water-cored apples and the adjacent foliage had a high concentration of sap and that this sap had a very high osmotic pressure. They also showed that fruit in the part of the tree most exposed to sun was most water cored. Artificial shading seemed to prevent water-core development.

Von Ende (*15*) states that a delicate balance exists between the enzyme processes which take place in the carbohydrate metabolism, that the process is reversible, and that the synthesis of starch and sucrose is a matter of the concentration of the hexoses. Sucrose is formed when the hexoses reach a certain concentration and starch is formed when the sucrose reaches a certain concentration.

Temperature readings of the foliage are presented in Table 9. The temperature of the leaf does not indicate perfectly the rate of transpiration, but the two are closely related. Each temperature given in the table represents an average of 60 to 130 determinations. These readings, taken in an orchard at Clarkston, Wash., with a thermocouple and galvanometer, indicate clearly that in the early part of the forenoon the foliage of the different lots gave off moisture at approximately the same rate. At midday the foliage attached to the spurs carrying fruit gave off moisture more rapidly than that attached to spurs of the same length without fruit. Evaporation of water from the foliage on twigs attached to spurs carrying fruit showed a slowing down very early in the day and continued at a slower rate than that on spurs without fruit until late in the day.

In Table 10 are shown the records for quality in Jonathan apples growing on spurs of different lengths in exposed and in shaded situations. These data represent quality as determined by taste only and have no reference to the appearance of the fruit as to color, size, or hardness. Each lot contained 100 apples. The fruit was about

average in range of hardness for the date of harvest and was from trees growing in a clean-tilled orchard. Fruit from an adjacent orchard carrying an alfalfa cover crop ranged practically the same in the different grades of quality.

TABLE 9.—*Leaf temperatures of Jonathan apple trees in orchard at Clarkston, Wash., September 12, 1925*

Lot No.	Type of spur	9 a. m.	11 a. m.	12.30 p. m.	2 p. m.	4 p. m.	5 p. m
	Air temperature in degrees Fahrenheit	70	81.0	85.0	89.0	82.0	75.0
1	Short nonfruiting	67	74.0	78.0	83.0	77.0	72.5
2	Short fruiting	67	73.6	77.5	83.7	76.0	71.7
3	Long nonfruiting	67	77.0	83.2	88.0	81.1	74.2
4	Long fruiting	67	75.1	81.5	87.0	80.0	73.0
5	Medium length nonfruiting with twig growth	67	74.0	82.0	86.7	80.1	74.0
6	Medium length fruiting with twig growth	67	74.0	81.0	85.1	79.8	73.4

TABLE 10.—*Quality of Jonathan apples harvested in Clarkston, Wash., September 10, 1926, as related to exposure to the sun*

[Tested Oct. 22, 1926]

Lot No.	Type of spur	Percentage of fruit grading—			
		Excellent	Good	Medium	Poor
	FRUIT AND FOLIAGE EXPOSED TO SUN				
1	Fruits attached to twigs or spurs over 18 inches long, without twig growth	0	3	9	88
2	Fruits attached to spurs 1 to 4 inches long, without twig growth	10	70	20	0
3	Fruits attached to spurs 8 to 12 inches long, without twig growth	8	32	51	9
4	Fruits attached to spurs 4 to 10 inches long, carrying short twig growth	41	42	12	5
	FRUIT AND FOLIAGE PARTIALLY OR ENTIRELY SHADED				
1	Fruits attached to twigs or spurs over 18 inches long, without twig growth	0	0	7	93
2	Fruits attached to spurs 1 to 4 inches long, without twig growth	0	7.5	41	51.5
3	Fruits attached to spurs 8 to 12 inches long, without twig growth	0	7	29	64
4	Fruits attached to spurs 4 to 10 inches long, carrying short twig growth	0	5	43	52

The fruits of highest quality were those well exposed to the sun and attached to relatively short, vigorous spurs on large branches or on longer spurs of sufficient vigor to produce a fruit and a twig at the same time. Fruit produced on long, slender twigs or spurs with few leaves and that grown on the long spurs, as lot 4 of Table 9, was of distinctly inferior quality.

The highest quality or highest flavored fruit was practically always found on the spurs which produced from the same winter bud a short, leafy twig, carrying 10 or more leaves, as lot 6, Table 9 and lot 4, Table 10, in contradistinction to those which produced the normal number of 5 to 7 leaves. It was noted also that maximum quality was quite uniformly found to exist in such fruit after three of four successive days of clear, dry weather, and that the lower quality of fruit was harvested from the same trees after a period of cloudy or rainy weather with lower temperatures.

High quality in apples seems to be closely related to medium growth and exposure to strong sunshine and dry air. Extreme drying of the foliage and fruit prevents functioning and injures quality. A constant high-moisture content of the plant provides for such perfect assimilation of the plant foods that there is only a medium or normal quantity stored in the fruit. The superior quality is developed under conditions between these extremes, that is, under such conditions as allow the maximum amount of sugars and other materials that improve flavor to be stored in the maturing fruit. This is probably one cause of the difference in quality of fruit from different districts, and also in different crops from the same orchard.

In two instances in 1924, duplicate samples of Jonathan apples were taken from the north and the south sides of the same tree and analyses were made. The fruit and adjacent foliage on the south side of the tree were exposed to the direct rays of the sun three to five hours longer each day than that on the north side, and the fruit showed more red color. No other recognizable difference in environment was apparent when the samples were gathered. These data are included in Table 4 under the dates of September 8 and 13. In both instances the total sugars, reducing sugars, alcohol-insoluble acid-hydrolyzable material, and total carbohydrates were higher in the samples taken from the south side. In one instance the sucrose was decidedly higher, while in the other it was a little lower in the sample from the south side. In both instances the insoluble material was lower in the samples taken from the south side of the tree.

Samples rating Fancy and C grade, 50 and 20 per cent, respectively, good red color, were taken on February 22 from a storage lot of Jonathan and one of Rome Beauty apples, and analysis made, with the results shown in Table 11. These results show that the sugars are higher in the Fancy than in the C grade apples. The difference in the amount of sucrose found in the two grades is rather striking. The percentage of acid, however, is practically the same. From these data it would therefore appear that for dessert quality in apples the quantity of sucrose present is more important than the quantity of acid.

TABLE 11.—*Composition of Fancy and C grade Jonathan and Rome Beauty apples taken from storage February 22*

Variety and grade of apple	Dry matter	Ash	Acid	Sugar		
				Reducing	Sucrose	Total
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Jonathan, fancy	15.96	0.256	0.45	8.98	2.32	11.30
Jonathan, C grade	15.24	.263	.41	8.54	1.21	10.15
Rome beauty, fancy	15.38	.185	.35	8.09	2.01	10.10
Rome beauty, C grade	12.44	.167	.27	6.13	1.32	8.25

Highest quality of the Jonathan, or the highest flavor of fruit, is found only in the highest colored fruits but is not uniform in proportion to red color. This has been found quite uniformly true of the fruit of the well-grown commercial crop. The fruits of medium size are usually of the best quality. This applies to the fruit that matures early, in midseason, or late on the tree. Time of harvest of

the material has apparently a direct relationship to quality in so far as it has a direct relationship to color attained by the fruit at the time of harvest.

The excellency of taste of apples bears the same relationship to color in fruit that is ripened or reaches its maximum dessert quality in storage as it does to that which reaches its maximum quality while on the trees. In the lower altitude, longer-growing-season districts of Washington, Jonathans are ripened and become of excellent quality on the tree, although the fruit may be removed from the tree, placed in storage, and have good storage life if harvested before the tissues have softened. The material grown in the higher altitude, shorter-season districts in some years will not reach the point of ripening on the trees, but must be harvested to avoid freezing before maximum quality or even full growth has been attained.

What is said in regard to color of the Jonathan apple applies equally to the Delicious, Winesap, and Rome Beauty. The Delicious showed the same variation as the Jonathan, although the formation and spur characteristics of the Delicious are quite different from those of the Jonathan.

In 1926, the Delicious crop on selected trees at Clarkston developed water-cored fruits before late harvest. The trees that were making poor growth, with very short twigs and relatively light foliage, developed fruit of medium size, high color, and medium to good quality with less than 5 per cent water core. Forty per cent of the fruits produced on trees making a medium growth, and with the crop thinned to a medium heavy load, were water-cored.

Water-cored fruit was more common on some classes of spurs than on others. On short spurs attached to large branches 10 per cent of the crop was water-cored; on long spurs attached to large branches 36 per cent was water-cored; while on medium-length spurs that had produced both fruit and twigs from the same winter bud, 70 per cent was water cored.

This distribution of water-cored material is more definite and clear-cut than any classification that could be made of the eating quality of the fruit attached to different twigs.

SUMMARY

The authors are unable to present at this time a system of measuring or defining the quality of apples that is accurate and practical for both the orchardist and the chemist.

Data on the composition and quality of Jonathan apples collected during several seasons are presented. A variation in duplicate samples from the same tree is noted, and this must be taken into consideration in interpreting results. Grade should be considered in taking samples, and also probably the position of the fruit on the tree.

The level of sugars and other fractions varies in different years. The dry matter, ash, nitrogen, and reducing sugars show no progressive variation during the growing season. The total sugars increase, due to an increase in sucrose.

The percentage of acid present shows a tendency to decrease during the growing season, but in view of the variations noted, it is doubtful whether much significance can be attached to the small change in

the quantity of acid. For dessert quality in apples the amount of sugar present seems to be of more importance than the amount of acid.

The decrease in the percentage of the alcohol-insoluble acid-hydrolyzable fraction during the growing season is more striking than the change in any other fraction. The constituents of this fraction should be studied further.

Such climatic factors as temperature, sunshine, and rainfall have a decided effect upon quality of fruit and the variation of quality from year to year. The taste of the apple as judged by different individuals varies, but it seems to be the most dependable basis which we have and the one upon which the trade must rely.

The best quality of apple is developed when the fruit and adjacent foliage are well exposed to sunshine and dry air. Conversely, shading the fruit and its adjacent foliage tends to retard quality development.

There is great variation in the quality of fruit produced by the trees in most of the orchards from which samples were taken, and the quality varies most in the trees carrying extremely dense foliage or casting heavy shade.

The method of pruning practiced in the apple orchards of Washington has a great influence over the quality and uniformity of the quality of the crop of apples produced.

Apples that reach full growth on the tree attain maximum quality as compared to those that are harvested before full size is attained.

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